1985

FACTORS CONTRIBUTING TO THE DEVELOPMENT OF FRAGILARIA CROTONENSIS KITTON "PULSES" IN THE PIGEON BAY WATERS OF WESTERN LAKE ERIE.

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FACTORS CONTRIBUTING TO THE DEVELOPMENT OF *FRAGILARIA CROTONENSIS* KITTON "PULSES" IN THE PIGEON BAY WATERS OF WESTERN LAKE ERIE

by

John Henry Hartig

A Dissertation

submitted to the Faculty of Graduate Studies

through the Department of

Biology in Partial Fulfillment

of the requirements for the degree

of Doctor of Philosophy at

The University of Windsor

Windsor, Ontario, Canada

1985

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ABSTRACT

In recent years, increased biomass of Fragilaria crotonensis has been reported from the Pigeon Bay waters of western Lake Erie for reasons not fully understood. This apparent increase in F. crotonensis biomass has been influenced primarily by large summer "pulses". The purpose of this study was to identify the factors which contribute to increased biomass of F. crotonensis in Pigeon Bay.

Both field and laboratory data suggest that F. crotonensis is eurythermal. However, maximum growth and photosynthetic rates of F. crotonensis in the laboratory occurred at warm temperatures (i.e. 17 and 23°C).

The early spring phytoplankton "pulse" begins under the ice when temperatures are low and nutrient concentrations relatively high. Fragilaria crotonensis is not a major component of this "pulse" because of a relatively high sinking rate causing its removal from the photic zone. Early on in spring turnover F. crotonensis appears to grow well because it is eurythermal, adapted to low light, and is a superior exploitative competitor for nutrients. As turnover continues depletion of silica results in silica limitation of F. crotonensis growth.

Summer not only provides optimal water temperatures for growth of F. crotonensis, but peak incident solar radiation and water transparency. It is hypothesized that the emergence of F. crotonensis "pulses" during summer is dependent upon three major factors: 1) adequate supplies of

nutrients (Si: via dissolution of biogenic silica and/or external loading; P: via luxury consumption and/or internal or external loading); 2) the presence of slight thermal stratification with concomitant wind velocities sufficient to keep F. crotonensis circulating in the surface mixed layer; and, 3) the presence of low turbidities with concomitant irradiance sufficient for growth. Once such physicochemical conditions exist in Pigeon Bay it appears that F. crotonensis can grow rapidly (because it is adapted to low light and is a superior exploitative competitor for nutrients) and achieve densities as high as 950,000 cells L^{-1}. Such "pulses" may be terminated by lack of wind (which allows cells to sink out of the photic zone), high wind velocities (which increase turbidity and decrease light penetration), or depletion of nutrients. Zooplankton grazing on F. crotonensis is probably insignificant because of its large size and chain-forming morphology.

In the fall in Pigeon Bay, F. crotonensis growth appears to be limited by light and silica supplies. Once water temperatures fall below 17°C, Pigeon Bay provides a suboptimal temperature environment for F. crotonensis growth.

In discussing the factors which contribute to F. crotonensis "pulses" in Pigeon Bay, one must maintain a historical perspective. For example, summer nitrate:soluble reactive phosphorus ratios have increased from approximately 4 in the late 1960's to over 40 in the late 1970's and early

1980's. During this same time period the summer phytoplankton shifted from being primarily nitrogen limited to being primarily phosphorus limited. Blue-green algal biomass declined while diatom biomass apparently increased. Fragilaria crotonensis appears to have become a more important component of the summer phytoplankton community in the late 1970's. It is possible that this increase in summer nitrate:soluble reactive phosphorus ratios in Pigeon Bay may provide a more optimal environment for F. crotonensis growth. Laboratory work has shown that F. crotonensis has a relatively high N:P ratio (25) which is consistent with its increased biomass with increased N:P ratios in Pigeon Bay.

ACKNOWLEDGEMENTS

I want to thank Drs. J.H. Leach, K.H. Nicholls, C.L. Schelske, P.D.H. Hebert, J.M. Winner, W.B. Drynan, and D.G. Wallen for their advice and guidance throughout this research effort. For assistance in laboratory work, I would like to thank Kerstin Hedgecock, Colleen Stein, Martha Morris Fathers, Betsy Rousseau, and Mary Landgraff. For taxonomic consultation and review of an earlier draft of Section 2, I would like to thank Gord Hopkins. Financial support was provided by Ontario Ministry of the Environment. Ontario Ministry of Natural Resources provided use of their boats. U.S. Environmental Protection Agency provided a water pollution control fellowship during 1982 and the University of Windsor provided graduate scholarships during 1983, 1984, and 1985.

Most of all I want to thank my wife and family for their encouragement and support throughout the course of my studies and research.

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1. General Introduction

Clearing of forest land for agriculture around 1850 resulted in the most significant disturbance to Lake Erie's metabolism (Harris and Vollenweider 1982). Since then at least parts of Lake Erie have exhibited mesotrophic to eutrophic status. The main evolution toward a eutrophic state occurred during the twentieth century. By the early 1960's Lake Erie was recognized as undergoing accelerated or cultural eutrophication (Beeton 1961).

Fragilaria crotonensis Kitton is often a predominant phytoplankter in eutrophic lakes (Wetzel 1983). It has historically been an important component of the phytoplankton of Lake Erie's western basin (Chandler 1944). Hohn (1969) reported densities of F. crotonensis remained fairly stable between 1938 and 1965 in the Bass Islands area of the western basin. However, relative abundance and volume of F. crotonensis declined substantially during that time. It was during the 1960's that blooms of blue-green algae became common in Lake Erie (Beeton 1969). Based on weekly phytoplankton samples collected from the Pigeon Bay waters of western Lake Erie via the Union Water Intake (Kingsville, Ontario), Nicholls et al. (1980) have reported that green and blue-green algal biomass declined between 1967 and 1978 due to reductions in phosphorus loading. During the same time period they reported increased F. crotonensis biomass for reasons not fully understood. This apparent increase in F. crotonensis biomass in Pigeon Bay

was primarily influenced by large summer F. crotonensis "pulses". The purpose of this study was to identify the factors which can contribute to increased biomass of F. crotonensis in Pigeon Bay. This was accomplished by investigating:

1) the seasonal variation of growth limiting nutrients in Pigeon Bay;
2) historical changes in nutrients in Pigeon Bay (including nitrate:soluble reactive phosphorus ratios);
3) the influence of light and temperature on growth and photosynthesis of axenic F. crotonensis from Lake Erie;
4) the influence of turbidity on Fragilaria biomass in Pigeon Bay;
5) the combined influence of light and nutrients on growth of F. crotonensis;
6) the role of sinking and resuspension of F. crotonensis cells in its seasonal succession; and,
7) the influence of zooplankton grazing on F. crotonensis periodicity in Pigeon Bay.

2. Seasonal Variation or Nutrient Limitation in Pigeon Bay

2.1 Introduction

It is generally accepted that in the western basin of Lake Erie during the late 1960's and early 1970's silica was the major nutrient limiting phytoplankton growth in the spring (Dobson et al. 1974; Thomas et al. 1979; DiToro 1980) and nitrogen was the major nutrient limiting phytoplankton growth in the summer (Lange 1971; Dobson et al. 1974; Schelske et al. 1978; Thomas et al. 1979; DiToro 1980; Murphy 1980). Lange (1971), Dobson et al. (1974), and Munawar and Burns (1976) also suggested that during that time western basin phytoplankton could have been periodically phosphorus limited.

In spite of the fact that western basin phytoplankton were predominantly nitrogen limited during summer, a phosphorus management strategy was implemented in the late 1960's (U.S. Public Health Service 1965) to control accelerated eutrophication because phosphorus is the most scarce and readily controllable nutrient relative to plant needs. As a result of the phosphorus control program, significant reductions in phosphorus loading to the western basin have occurred (IJC 1981; Hartig and Horvath 1982; Hartig 1983). The western basin has responded with lower phosphorus concentrations and a decline in phytoplankton (Nicholls et al. 1980; IJC 1981). The purpose of this study was to identify nutrients limiting growth of natural phytoplankton assemblages in light of the chemical and

biological changes which have occurred in the western basin.

2.2 Materials and Methods

Water samples containing natural phytoplankton were collected monthly from April to October, 1983, from a station at 42°02'53"N and 82°41'00"W in the Pigeon Bay waters of the western basin. This station is approximately 0.5 km off the north shore at a depth of 4.5 m. It is also located in the vicinity of the Union Water Intake of Kingsville, Ontario from which western basin water quality has been monitored since 1967 (Nicholls et al. 1980). Water samples were collected from 1.5 m using an opaque plastic, Van Dorn bottle and immediately transferred to a 10-L polyethylene bottle. During transport, water samples were kept in the dark and maintained at ambient water temperatures. All samples were delivered to the laboratory within 2 hours.

Immediately upon arrival at the laboratory, the lake water sample was thoroughly mixed and three 100 ml subsamples were filtered through separate Whatman GF/C filters which were then extracted with 90% acetone for chlorophyll a analyses using the fluorometric method (Turner Model 430 Spectrofluorometer) of Strickland and Parsons (1968). Additional lake water was filtered through a 47 mm Millipore filter (0.45 µm pore size) and the filtrate analyzed for soluble reactive and total soluble (ultraviolet light oxidation) phosphorus (Wetzel and Likens 1979), nitrate (Golterman 1969), and reactive silicate (Golterman

1969) using a Hitachi Perkin-Elmer, Coleman 111 Spectrophotometer. Three 100 ml subsamples of lake water were also preserved in Lugol's solution for phytoplankton identification and enumeration using an inverted microscope (Utermöhl 1958).

For each nutrient enrichment experiment, 250 ml of untreated lake water were dispensed into each of 36 numbered 500 ml polycarbonate Erlenmeyer flasks. These flasks were divided into 12 three-flask sets with each set receiving one of the following treatments: control (distilled water), phosphorus (+P), silica (+Si), nitrogen (+N), trace metals (+TM), vitamins (+VIT), complete (which included phosphorus, silica, nitrogen, trace metals, and vitamins), complete minus phosphorus (-P), complete minus silica (-Si), complete minus nitrogen (-N), complete minus trace metals (-TM), and complete minus vitamins (-VIT) (Table 1). Treatments were made by dispensing 0.25 or 0.5 ml aliquots of separately prepared stock solutions directly into the flasks. Thiamine, cyanocobalamin, and biotin were added as one treatment (vitamins) and Fe, Mo, B, Cu, Zn, Co, and Mn were combined with EDTA as another treatment (trace metals) at concentrations commonly employed in media used to culture phytoplankton (Guillard and Lorenzen 1972). After treatment, samples were placed in an environmental chamber and incubated for 9 days, during which each flask was shaken daily by hand and repositioned at random.

In the environmental chamber, light:dark cycles and

Table 1. Concentrations of major nutrients, vitamins, trace metals, and EDTA used in nutrient enrichment experiments.

Nutrient	Concentration ($\mu g\ L^{-1}$)	Compound
P	25	KH_2PO_4
N	250	$NaNO_3$
Si	500	$Na_2SiO_3 \cdot 9H_2O$
Vitamins (VIT)		
B_1	500	Thiamine-HCl
B_{12}	2	Cyanocobalamin
Biotin	1	Biotin
Trace Metals (TM)		
Fe	650	$FeCl_3 \cdot 6H_2O$
Mo	2.5	$Na_2MoO_4 \cdot 2H_2O$
B	170	H_3BO_3
Cu	2.5	$CuSO_4 \cdot 5H_2O$
Zn	5.0	$ZnSO_4 \cdot 7H_2O$
Co	2.5	$CoCl_2 \cdot 6H_2O$
Mn	0.05	$MnCl_2 \cdot 4H_2O$
EDTA	3373	$Na_2EDTA \cdot 2H_2O$

temperatures were programmed to simulate natural seasonal variations (Table 2). Environmental chamber light intensities of 150 $\mu E\ m^{-2}\ s^{-1}$ for summer and 120 $\mu E\ m^{-2}\ s^{-1}$ for the remainder of the year were established using 20-W cool white fluorescent tubes. Light intensities were measured with a LI-COR LI-193SB spherical quantum sensor which measures photosynthetically active radiation (400-700 nm).

Changes in chlorophyll a concentration were used to evaluate the growth response of phytoplankton to nutrient enrichments. Schelske (1984) has shown the efficacy of this technique in assessing nutrient limitation. Chlorophyll a concentrations were measured in 50 ml subsamples taken from each flask at days 3, 6, and 9. Mean daily growth rates over the incubation period were calculated for each treatment from the equation

$$K = \frac{\ln N_2/N_1}{t_2 - t_1}$$

where N_1 and N_2 represent chlorophyll a concentration ($\mu g\ L^{-1}$) at days 1 (t_1) and 6 or 9 (t_2).

Analysis of variance procedures were used to determine if nutrient enrichment did significantly affect growth rate. Duncan's multiple range tests were used to determine which treatments resulted in significantly different growth rates (Zar 1974).

2.3 Results

Table 2. Environmental chamber temperatures, light:dark cycles, and light intensities used for nutrient enrichment experiments.

Date (1983)	Temperature (^{o}C)	Light:Dark (hours)	Light Intensity (μE $m^{-2}s^{-1}$)
27 Apr.	5-7	12:12	120
17 May	7-9	14:10	120
21 June	19-21	15:9	150
26 July	22-24	14.5:9.5	150
15 Aug.	22-24	14:10	150
26 Sept.	16-18	12:12	150
26 Oct.	11-13	11:13	120

2.3.1 Seasonal Periodicity of Nutrients and Phytoplankton in Pigeon Bay

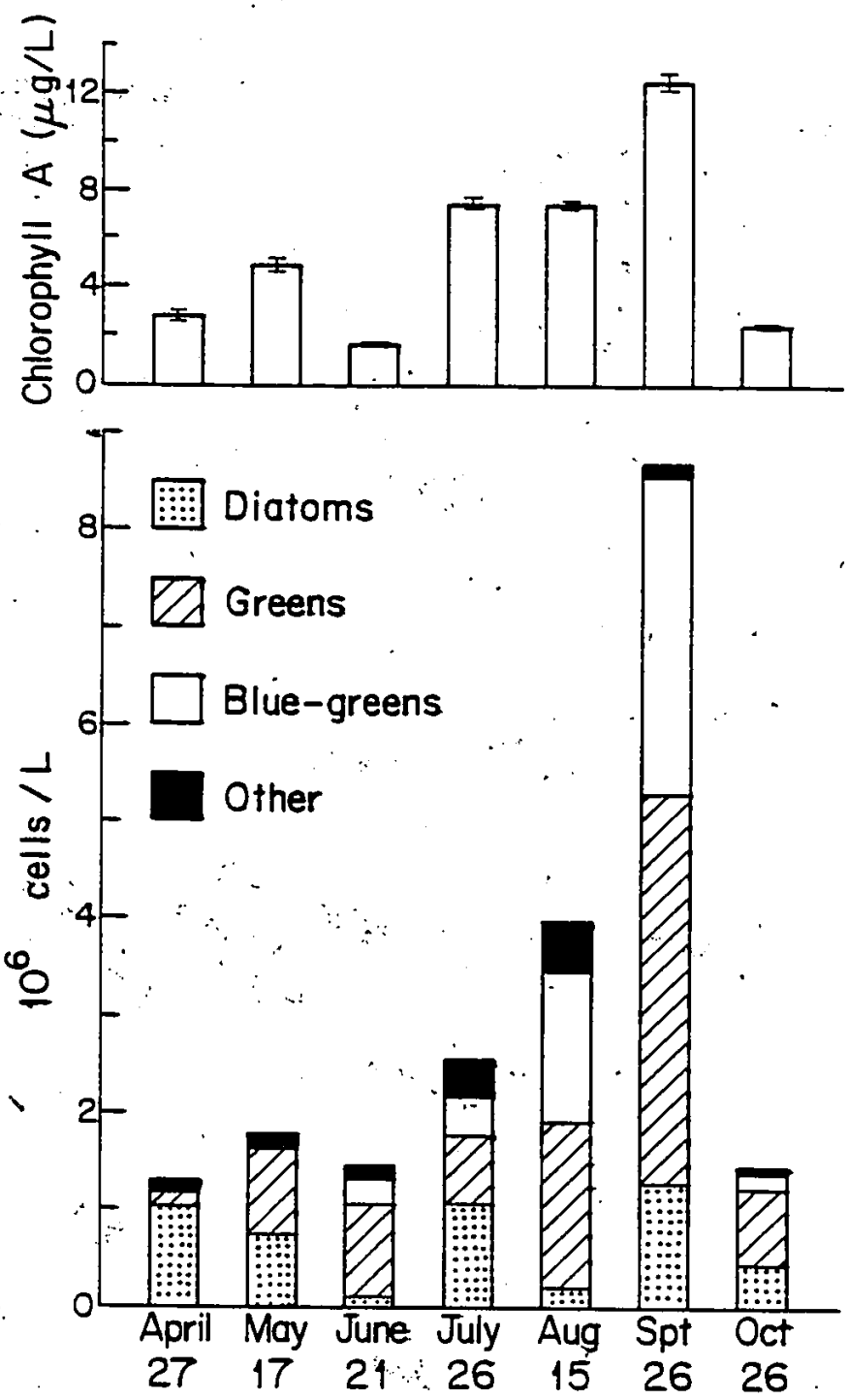
In April and May, concentrations of reactive silicate at the Pigeon Bay station were depleted to less than 20 μg L^{-1} (Table 3). Reactive silicate started to increase in June and peaked in July and August at concentrations exceeding 660 μg L^{-1}. In September and October reactive silicate decreased to approximately 200 μg L^{-1}. Nitrate concentrations remained relatively high (> 210 μg L^{-1}) throughout the sampling program with the exception of July, August, and September when they decreased to 120-180 μg L^{-1}. Soluble reactive phosphorus concentrations were consistently low throughout the sampling program ranging from below detection limits to 5.5 μg L^{-1}. In contrast, total soluble phosphorus concentrations were relatively high, varying from 43.2 to 67.1 μg L^{-1}.

Seasonal periodicity of chlorophyll a and phytoplankton cell counts in Pigeon Bay followed similar seasonal patterns with a small peak in May, a minimum in June, a progressive increase from July to September, and then a dramatic decrease in October (Figure 1). In April the phytoplankton community was 80 % diatoms (predominant genera: Asterionella and Fragilaria), followed by increased proportions of green algae in May (predominant genera: Chlamydomonas and Monoraphidium) and June (predominant group: unicellular greens), a July diatom pulse (predominant genus: Fragilaria), and then a shift in

Table 3. Mean (n=2) nutrient concentrations in Pigeon Bay at the outset of nutrient enrichment experiments, April-October, 1983.

Date (1983)	Soluble Reactive Phosphorus ($\mu g\ PO_4\text{-}P\ L^{-1}$)	Total Soluble Phosphorus ($\mu g\ PO_4\text{-}P\ L^{-1}$)	Reactive Silicate ($\mu g\ Si\ L^{-1}$)	Nitrate Nitrogen ($\mu g\ N\ L^{-1}$)
27 Apr.	1.5	45.2	13.9	277.0
17 May	4.6	54.9	13.4	215.7
21 June	< 1.0	58.4	75.1	370.1
26 July	5.5	46.5	871.4	171.1
15 Aug.	< 1.0	62.1	665.1	182.6
26 Sept.	< 1.0	67.1	161.0	123.1
26 Oct.	4.6	43.2	227.1	218.8

Figure 1. Seasonal periodicity of chlorophyll a (top) and phytoplankton densities (bottom) in Pigeon Bay. Chlorophyll a data are presented as means of triplicate measurements with error bars indicating plus and minus one standard deviation.


Chlorophyll A (μg/L)
0
4
8
12
Diatoms
Greens
Blue-greens
Other
10^6 cells / L
0
2
4
6
8
April 27
May 17
June 21
July 26
Aug 15
Spt 26
Oct 26

dominance to blue-green algae in August and September (predominant genera: Microcystis and Merismopedia). September also exhibited a diatom pulse (predominant genera: Melosira and Fragilaria). October phytoplankton were dominated by green algae (predominant genus: Scenedesmus).

2.3.2 Nutrient Enrichment Experiments

Highest chlorophyll a concentrations (Figure 2) and phytoplankton growth rates in chlorophyll a doublings day^{-1} (Table 4) occurred usually in complete treatments while lowest chlorophyll a concentrations and phytoplankton growth rates occurred usually in control treatments. In most cases multiple nutrient enrichment treatments (complete, -P, -Si, -N, -TM, -VIT) resulted in higher chlorophyll a yields than did single nutrient enrichment treatments (+P, +Si, +N, +TM, +VIT). Maximum chlorophyll a concentrations (approximately 50 µg L^{-1}) were observed in the July complete treatments which represented a 700 percent increase from initial in-lake concentrations.

In April, May, and October highest chlorophyll a concentrations were found on day 9 of the experiments (Figure 2). In June, July, August, and September highest chlorophyll a concentrations were found on day 6, followed by a decrease at day 9. To assess nutrient limitation, phytoplankton growth rates in chlorophyll a doublings day^{-1} were calculated using initial and day 9 data in April, May, and October and initial and day 6 data in June-September (Table 4). Analysis of variance procedures performed on

Figure 2. Seasonal variation of the effect of nutrient enrichments on chlorophyll *a* concentration. Data are presented as means of triplicate experiments with error bars indicating plus and minus one standard deviation.

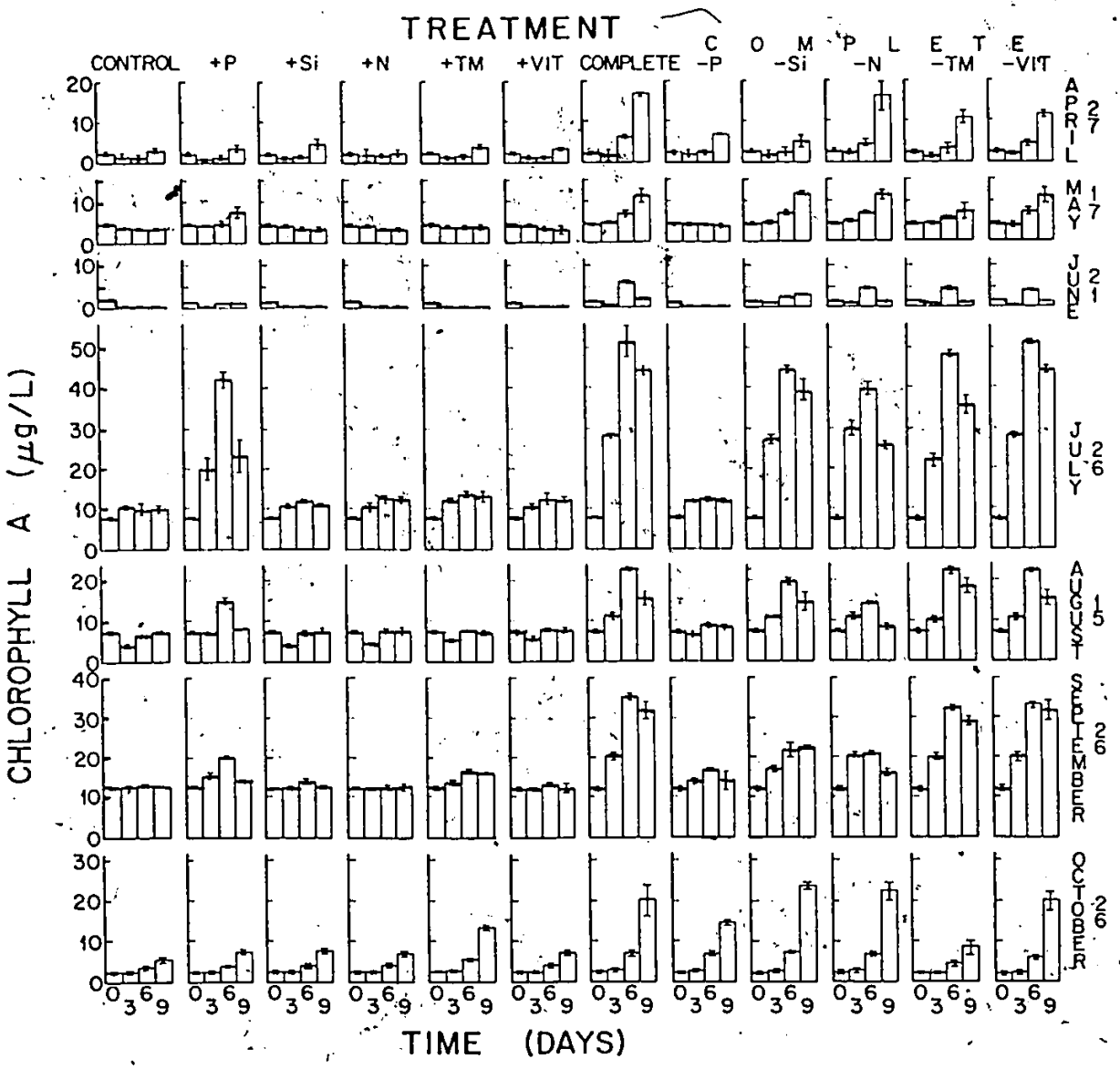

TREATMENT
CONTROL
+P
+Si
+N
+TM
+VIT
COMPLETE
COMPLETE -P
COMPLETE -Si
COMPLETE -N
COMPLETE -TM
COMPLETE -VIT
APRIL 27
MAY 17
JUNE 21
JULY 26
AUGUST 15
SEPTEMBER 26
OCTOBER 26
CHLOROPHYLL A (µg/L)
TIME (DAYS)

Table 4. Seasonal variation of phytoplankton growth rates (chlorophyll *a* doublings day^{-1}) observed in nutrient enrichment experiments with corresponding Duncan's multiple range test groupings ($P<0.05$). Means with the same letter are not significantly different. Growth rates were measured over 9 days in April, May, and October and over 6 days in June-September.

27 APRIL 1983

Treatment	Mean Growth Rate	Duncan's Multiple Range Test
Complete	0.201	A
-N	0.197	A
-VIT	0.158	B
-TM	0.148	B
-P	0.096	C
-Si	0.070	D
+Si	0.046	E
+TM	0.041	E
+P	0.030	E F
+VIT	0.024	E F
Control	0.015	F
+N	0.014	F

27 MAY 1983

Treatment	Mean Growth Rate	Dupcan's Multiple Range Test
-Si	0.100	A
-N	0.097	A
-VIT	0.096	A
Complete	0.092	A
-TM	0.045	B
+P	0.045	B
-P	0.000	C
Control	0.000	C
+VIT	0.000	C
+Si	0.000	C
+N	0.000	C
+TM	0.000	C

21 JUNE 1983

Treatment	Mean Growth Rate	Duncan's Multiple Range Test
Complete	0.219	A
-TM	0.179	B
-N	0.179	B
-VIT	0.145	C
-Si	0.070	D
+P	0.000	E
-P	0.000	E
Control	0.000	E
+VIT	0.000	E
+Si	0.000	E
+N	0.000	E
+TM	0.000	E

26 JULY 1983

Treatment	Mean Growth Rate	Duncan's Multiple Range Test
Complete	0.321	A
-VIT	0.317	A
-TM	0.313	A
-Si	0.298	A B
+P	0.289	B
-N	0.277	B
+TM	0.100	C
+N	0.092	C D
+VIT	0.087	C D
-P	0.085	C D
+Si	0.073	D
Control	0.034	E

15 AUGUST 1983

Treatment	Mean Growth Rate	Duncan's Multiple Range Test
Complete	0.187	A
-TM	0.186	A
-VIT	0.168	B
-Si	0.164	B
+P	0.119	C
-N	0.112	C
-P	0.037	D
+VIT	0.016	E
+TM	0.009	E F
+N	0.006	F
+Si	0.001	F
Control	0.000	F

26 SEPTEMBER 1983

Treatment	Mean Growth Rate	Duncan's Multiple Range Test
Complete	0.169	A
-VIT	0.161	A
-TM	0.155	A
-Si	0.091	B
-N	0.086	B C
+P	0.077	C
-P	0.052	D
+TM	0.031	E
+Si	0.021	E F
+VIT	0.014	F G
Control	0.008	F G
+N	0.006	G

26 OCTOBER 1983

Treatment	Mean Growth Rate	Duncan's Multiple Range Test
-Si	0.255	A
-N	0.248	A
-VIT	0.239	A
Complete	0.236	A
-P	0.199	B
+TM	0.193	B
-TM	0.148	C
+Si	0.127	D
+VIT	0.123	D
+P	0.122	D
+N	0.121	D
Control	0.095	E

these data showed that nutrient enrichment did significantly (α level: .0001) affect phytoplankton growth during each month (April-October).

In April, highest mean chlorophyll *a* concentrations were produced on day 9 in complete and -N treatments (17.0 and 16.6 $\mu g\ L^{-1}$, respectively; Figure 2). Of the single nutrient enrichment treatments +Si produced the highest mean chlorophyll *a* concentration (4.3 $\mu g\ L^{-1}$). Results from Duncan's multiple range test showed that even though +Si produced the highest growth rates ($\bar{x}$= 0.046 chlorophyll *a* doublings day^{-1}) of the single nutrient enrichment treatments, it was not significantly different from +TM, +P, and +VIT treatments (Table 4). However, of the multiple nutrient enrichment treatments -Si had the lowest growth rates ($\bar{x}$= 0.070 chlorophyll *a* doublings day^{-1}) and was significantly different from the other multiple nutrient enrichment treatments. This suggests that silica was probably the nutrient most limiting phytoplankton growth in April.

In May, only treatments containing phosphorus exhibited an increase in chlorophyll *a* concentration over the 9 day experiment (Figure 2). In these treatments, mean phytoplankton growth rates ranged from 0.045 chlorophyll *a* doublings day^{-1} in +P and -TM treatments to 0.100 chlorophyll *a* doublings day^{-1} in complete treatments. All treatments containing phosphorus had significantly higher growth rates than treatments without phosphorus which

exhibited no growth (Table 4), suggesting that phosphorus was the nutrient most limiting phytoplankton growth in May.

In June, a Mougeotia sp. - Lyngbya sp. assemblage was observed growing from days 6 to 9 on the sides of all flasks of treatments containing phosphorus. This growth was absent in flasks not enriched with phosphorus. Chlorophyll a data up to day 6 show that growth only occurred in -Si, -N, -TM, -VIT, and complete treatments (Figure 2). No growth occurred in the -P treatment and this was obviously not significantly different from the control, +Si, +N, +TM, +VIT, and +P treatments (Table 4). These observations, although not conclusive, suggest that phosphorus was probably the nutrient most limiting phytoplankton growth in June.

In July, August, and September highest chlorophyll a concentrations were produced on day 6 in all treatments containing phosphorus (Figure 2). A decrease in chlorophyll a was also noted in these treatments from days 6 to 9. In treatments containing phosphorus, the ranges of mean phytoplankton growth rates in July, August, and September were 0.277-0.321, 0.112-0.187, and 0.077-0.169 chlorophyll a doublings day^{-1}, respectively. All treatments with phosphorus had higher phytoplankton growth rates and were significantly different from all treatments without phosphorus (Table 4), suggesting that phosphorus was the nutrient most limiting phytoplankton growth in July, August, and September.

In October, highest chlorophyll a concentrations were produced on day 9 of the experiment in all treatments containing trace metals (Figure 2). In these treatments, mean chlorophyll a doublings day^{-1} ranged from 0.193 in +TM treatments to 0.255 in -Si treatments (Table 4). All treatments containing trace metals had higher phytoplankton growth rates and were significantly different from all treatments without trace metals, suggesting that trace metals limited phytoplankton growth most in October.

2.3.3. Nutrient Trends In Pigeon Bay

Since 1967, water quality monitoring in Pigeon Bay has been performed by provincial and federal government agencies. The Great Lakes Surveys Unit of Ontario Ministry of the Environment (OME) monitored selected parameters including soluble reactive phosphorus and nitrate-nitrogen at 10-20 stations in the nearshore waters of Pigeon Bay from 1967-1975 (Y. Hamdy, OME, 1984). The Lake Erie Fisheries Research Station of Ontario Ministry of Natural Resources (OMNR) monitored selected parameters including soluble reactive phosphorus, nitrate-nitrogen, and reactive silicate at three stations in the nearshore waters of Pigeon Bay from 1971-1975 (J.H. Leach, OMNR, 1984). The Limnology and Toxicity Section of OME has performed weekly monitoring since 1976 of selected parameters including soluble reactive phosphorus, nitrate-nitrogen, and reactive silicate at the Union Water Intake which draws water from Pigeon Bay (K.H. Nicholls, OME, 1984). These three data bases were combined

into one which is presented to evaluate trends (Figure 3).

Even though these trend data are open to criticism because three different laboratories performed analyses, laboratory techniques have improved, and stations sampled have differed from year to year they are the only trend data available for these parameters. High variability of the data presented is believed to be representative of the dynamic nature of the western basin (Chandler and Weeks 1945). Seasonal patterns of reactive silicate have remained fairly comparable with lowest concentrations normally found in spring and sometimes winter (Figure 3a). Comparatively low reactive silicate concentrations in spring have also been reported in the western basin by Dobson et al. (1974), Gächter et al. (1974), and DiToro (1980). Nitrate concentrations in Pigeon Bay have steadily increased since the late 1960's (Figure 3b). For example, mean summer nitrate concentrations have increased from < 100 $\mu g\ L^{-1}$ in the late 1960's and early 1970's to > 200 $\mu g\ L^{-1}$ in the early 1980's. A one-way analysis of variance showed that time (year) had a significant (α level: .0001) effect on nitrate concentration during July-September in Pigeon Bay. Duncan's multiple range test groupings show that during 1977 and 1979-1983 summer nitrate concentrations in Pigeon Bay were significantly higher than 1967-1976 and 1978 (Table 5). Comparatively low summer nitrogen concentrations during the late 1960's and early 1970's in the western basin have also been reported by Dobson et al. (1974), Gächter et al.

Figure 3. Nutrient trends in the Pigeon Bay waters of western Lake Erie, 1967-1983. A, reactive silicate (RS) concentrations (μg Si L^{-1}); B, nitrate concentrations; C, soluble reactive phosphorus (SRP) concentrations (μg PO_4-P L^{-1}); D, nitrate:soluble reactive phosphorus ratios. Data are presented as means with error bars indicating plus and minus one standard error (○ = January-March; ◆ = April-June; ● = July-September; ◇ = October-December). Sources of data: OME and OMNR.

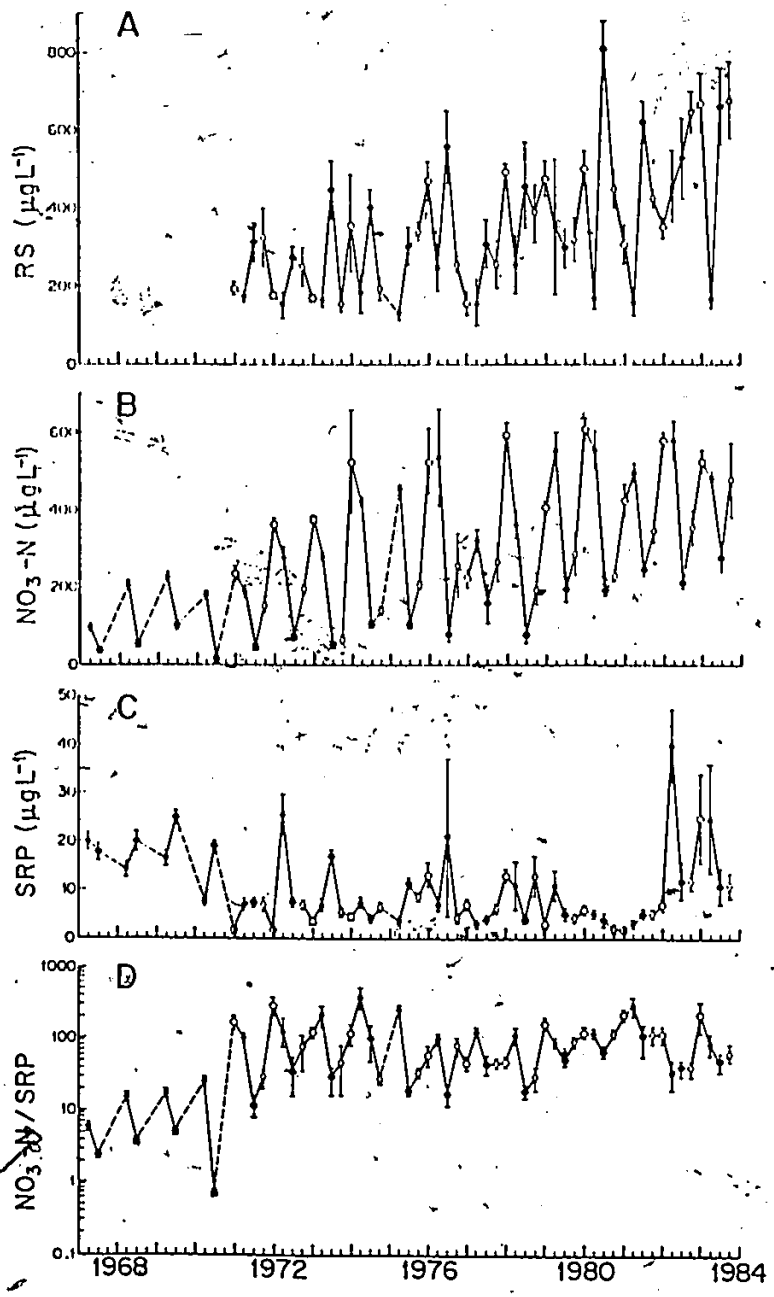

A
RS (µgL⁻¹)
800
600
400
200
0
B
NO3-N (µgL⁻¹)
600
400
200
0
C
SRP (µgL⁻¹)
50
40
30
20
10
0
D
NO3-N / SRP
1000
100
10
1
0.1
1968
1972
1976
1980
1984

Table 5. Mean summer (July-September) nitrate concentrations, soluble reactive phosphorus (SRP) concentrations, and nitrate:soluble reactive phosphorus ratios in Pigeon Bay, 1967-1983. Duncan's multiple range tests were used to determine which years were significantly defferent ($P<0.05$). Means with the same letter are not significantly different.

Nitrate			Soluble Reactive Phosphorus			Nitrate:SRP Ratio		
Year	Mean Concentration ($\mu g\ L^{-1}$)	Duncan's Multiple Range Test	Year	Mean Concentration ($\mu g\ L^{-1}$)	Duncan's Multiple Range Test	Year	Mean Concentration ($\mu g\ L^{-1}$)	Duncan's Multiple Range Test
1983	276.9	A	1969	24.7	A	1981	75.6	A
1981	243.9	AB	1968	19.7	AB	1980	66.5	AB
1982	216.2	BC	1970	18.9	B	1979	54.7	BC
1979	193.9	CD	1967	17.6	B	1983	53.6	BCD
1980	190.0	CD	1973	16.8	BC	1977	46.0	CD
1977	162.3	D	1982	11.5	CD	1982	42.4	CD
1974	111.6	E	1983	10.8	DE	1974	36.3	D
1975	108.5	E	1975	9.8	DEF	1975	19.5	E
1969	106.9	E	1971	7.3	DEF	1978	19.2	E
1976	80.0	EF	1972	7.3	DEF	1976	18.8	E
1978	78.3	EF	1981	5.1	EF	1972	16.1	E
1972	73.4	EF	1979	5.1	EF	1971	9.6	E
1968	56.7	F	1974	4.5	F	1973	9.4	E
1973	53.0	FG	1980	4.3	F	1969	5.2	E
1971	42.6	FG	1976	4.3	F	1968	3.3	E
1967	38.4	FG	1978	4.2	F	1967	2.5	E
1970	13.5	G	1977	4.2	F	1970	0.7	E

(1974), and DiToro (1980). In general, soluble reactive phosphorus concentrations have decreased since the late 1960's, yet periodic high concentrations can still occur as evidenced by values exceeding 20 $\mu g\ L^{-1}$ in 1982 and 1983 (Figure 3c). A one-way analysis of variance showed that time (year) also had a significant (α level: .0001) effect on soluble reactive phosphorus concentration during summer in Pigeon Bay. Duncan's multiple range test showed that summer soluble reactive phosphorus concentrations in Pigeon Bay during 1967-1970 and 1973 were significantly higher than during 1971-1972 and 1974-1983 with the exception of 1973 which was not significantly different from 1982 (Table 5). Comparatively high soluble reactive phosphorus concentrations (>20 $\mu g\ L^{-1}$) in the late 1960's and early 1970's in the western basin have also been reported by Dobson et al. (1974), Gächter et al. (1974), DiToro (1980), and Schelske et al. (1978). The combination of a general decrease in soluble reactive phosphorus concentrations and increased nitrate concentrations has caused a significant increase in mean summer nitrate-soluble reactive phosphorus ratios of an order of magnitude (Figure 3d; Table 5).

2.4 Discussion

2.4.1 Seasonal Variation of Nutrient Limitation

Growth of a single algal species at any given time can only be limited by a single nutrient (Rhee and Gotham 1980). However, different species within a phytoplankton community may be limited by different nutrients because of varying

abilities to acquire and utilize nutrients (Tilman 1977). In addition, nutrient ratios are believed to play important roles in structuring phytoplankton communities (Tilman et al. 1982). In my nutrient enrichment experiments growth (as evidenced by increases in chlorophyll a concentration) was highest in multiple nutrient enrichment treatments. This is not surprising because my experiments utilized natural phytoplankton assemblages where part of the assemblage (i.e. one species or taxonomic group) may be responding to one nutrient while another part may be responding to a different nutrient. In April, May, and September there were obvious synergistic effects in multiple nutrient enrichment treatments where, for example, the growth rate in complete treatments was much higher than the sum of the growth rates of single nutrient enrichment treatments. In other cases there were no obvious synergistic effects and growth in all treatments was affected predominantly by the presence or absence of one nutrient (e.g. in July by phosphorus). Synergistic effects may have been the result of changes in nutrient ratios or interactions of light and nutrients. Rhee and Gotham (1981) have shown that the combined effects of light and nutrient on phytoplankton growth are greater than the sum of individual effects.

In April when the phytoplankton community was 80 % diatoms and reactive silicate concentration was less than 20 $\mu g\ L^{-1}$ the nutrient enrichment experiment results provided

good evidence that silica was the primary limiting nutrient. Springtime silica limitation in the western basin has been inferred several times by low reactive silicate concentrations, which are believed to reflect utilization by diatoms (Dobson et al. 1974; Thomas et al. 1979; DiToro 1980). Schelske and Stoermer (1971) have indicated that during intensive diatom blooms silica is limiting at concentrations less than 100 μg SiO_2 L^{-1}. The Pigeon Bay trend data from 1971-1983 suggest that low silica concentrations in spring is a common occurrence in these waters. The results of the April experiment also showed slight enhancement of growth in +P and +TM treatments which indicates that phosphorus and a trace metal may have been secondary limiting nutrients.

In May, reactive silicate concentrations were still low (< 20 μg L^{-1}) yet the results indicate that phosphorus was the primary nutrient limiting phytoplankton growth. The probable reason for this is that the phytoplankton community structure shifted from being dominated by the silica requiring diatoms in April to being dominated by green algae in May.

In June, July, August, and September the results indicate that phosphorus was the primary limiting nutrient. In three of these months there was no detectable soluble reactive phosphorus; in July soluble reactive phosphorus was 5.5 μg L^{-1}. Lean et al. (1983) have shown that with depletion of soluble reactive phosphorus the phytoplankton

in the eastern and central basins of Lake Erie become phosphorus limited. Data from my enrichment experiments support their conclusion and may be interpreted to suggest that phosphorus concentrations of at least 5.5 $\mu g\ L^{-1}$ can be limiting to the phytoplankton community in Pigeon Bay. In addition, even though soluble organic phosphorus (total soluble minus soluble reactive phosphorus) exceeded 40 $\mu g\ L^{-1}$ during these months this phosphorus fraction was apparently not available to the phytoplankton.

The western basin is shallow (mean depth: 7.6 m) and is exposed to strong winds that make holomixis a common feature of the basin. Because holomixis is common, resuspension of phosphorus via mixing of the water column is believed to be significant to the basin's metabolism (Chapra 1982). If this resuspended phosphorus is bioavailable, it may periodically relieve phosphorus limitation. One might think that periodic contact with sediments or resuspension of phosphorus would maintain adequate internal phosphorus pools in phytoplankton and avoid phosphorus limitation. Indeed, luxury consumption of phosphorus has been shown to sustain phytoplankton during periods of low availability (Lean et al. 1983). However, the results of this study show that Pigeon Bay phytoplankton can be phosphorus limited. In Saginaw Bay (Lake Huron) Bierman et al. (1984) concluded that particulate phosphorus from resuspended sediments was probably not in a form immediately available to phytoplankton and that the average residence time in the

water column was not sufficient for conversion of a substantial amount to an available form. It remains possible that because of the dynamic and productive nature of the western basin, phosphorus limitation can potentially be relieved by sufficient internal loading of bioavailable phosphorus.

In 1976 Lean and Nalewajko (1979) studied an open-water station in the western basin where the mixing depth (10 m) exceeded the depth of light penetration (1% light: 2.2 m), hence, phytoplankton were being mixed down to 10 m and consequently spent less time in light than if the entire mixing zone was illuminated. They suggest that under these conditions western basin phytoplankton growth was light limited and that this contributed to a low phosphorus demand, as indicated by a phosphate turnover time of 12 hours. Furthermore, it has been suggested that nutrients excreted by zooplankton (Lean et al. 1982) or remineralized by bacteria (McCarthy and Goldman 1979) may help relieve nutrient limitation of adjacent phytoplankton cells. Contact with sediments at the bottom or resuspended material may offer phytoplankton a phosphorus source not yet quantified. Rhee and Gotham (1981) have reported simultaneous limitations of light and nutrient on growth of phytoplankton. This may be precisely what surface water phytoplankton face in the western basin (i.e. simultaneous light and nutrient limitation). However, it would be very difficult to determine the frequency of nutrient and/or

light limitation because the western basin is shallow and therefore particularly subject to unpredictable meteorological conditions.

The results of this study indicate that nitrogen may have been a secondary limiting nutrient in August and September when mean nitrate concentrations were 182 and 123 $\mu g\ L^{-1}$, respectively, and the phytoplankton community was approximately 40% blue-green algae. It is also possible that the increase in chlorophyll *a* concentration resulting from nitrogen enrichment may not reflect an increase in phytoplankton growth but an increase in chlorophyll content of cells. Nicholls and Dillon (1978) have concluded that nitrogen availability can be a major factor controlling chlorophyll content of algal cells.

In October, trace metals limited phytoplankton growth most. Unfortunately, because of the experimental design, I cannot tell which trace metal was limiting. Certain trace metals are essential to phytoplankton and, under certain conditions of restricted availability, can limit phytoplankton growth (Lange 1971). One possible explanation for how trace metal limitation could occur in the western basin is that the high standing crop of phytoplankton in September could have decreased trace metal concentrations to levels limiting phytoplankton growth in October. Adsorption of trace metals onto suspended solids may also contribute to low availability of a trace metal in the western basin. Lange (1971) reported cobalt and chelated iron limiting

phytoplankton growth in one-third of his western basin enrichment experiments in 1969.

These enrichment experiments clearly show that nutrient limitation of phytoplankton is a complex and dynamic phenomenon. Wetzel (1983) believes that limitation can shift rapidly from nutrient to nutrient as their availabilities change on a diurnal, daily, and seasonal basis. These results show that nutrient enrichment experiments should be performed often enough to account for seasonal changes in the physicochemical environment as well as seasonal succession in phytoplankton.

2.4.2 Shift in Nutrient Limitation

Based on monthly nutrient enrichment experiment data from May through September, 1983, phosphorus was the primary nutrient limiting phytoplankton growth at the Pigeon Bay station. This conclusion is different from the conclusion that in the late 1960's and early 1970's nitrogen was probably the primary nutrient limiting phytoplankton growth in summer in the western basin and suggests a possible shift from nitrogen to phosphorus limitation. The Pigeon Bay trend data provide evidence that a shift has probably occurred from a primarily nitrogen limited state in summer to a phosphorus limited state. Prior to 1970, Pigeon Bay frequently had high soluble reactive phosphorus concentrations, very low nitrate concentrations in summer, and low summer nitrate-soluble reactive phosphorus ratios suggesting that nitrogen was probably the major nutrient

limiting phytoplankton growth in summer. Schelske et al. (1978) have concluded that chemical measurements can be substituted for nutrient enrichment experiments in assessing nutrient limitation under certain conditions (e.g. high soluble reactive phosphorus concentrations in the late 1960's in the western basin would not limit phytoplankton growth while low nitrogen concentrations could). In the late 1970's and early 1980's, Pigeon Bay generally had lower soluble reactive phosphorus concentrations, higher nitrate concentrations in summer, and mean summer nitrate-soluble reactive phosphorus ratios > 40 suggesting that nitrogen was probably not limiting based on the nutrient ratio work of Rhee (1978), Rhee and Gotham (1980), Chiaudani and Vighi (1974), and Forsberg et al. (1978).

Evaluating trends of nutrients in the western basin is complicated because holomixis is a common feature of the basin which can result in significant internal loading of nutrients (particularly phosphorus) from sediments (Lam and Jaquet 1976). Considering that holomixis is common in the western basin and that approximately 80 percent of the external phosphorus loading to Lake Erie becomes incorporated in sediments (IJC 1981), episodes of high internal phosphorus loading might periodically result in high soluble reactive phosphorus concentrations that may relieve phosphorus limitation. The Pigeon Bay trend data demonstrate the variability of soluble reactive phosphorus yet also show a general decrease in concentration during the

July-August period from approximately 20 $\mu g\ L^{-1}$ in the late 1960's to approximately 10 $\mu g\ L^{-1}$ or less in recent years.

This shift from Pigeon Bay's being primarily nitrogen limited in summer to being primarily phosphorus limited in summer is probably the result of the combination of reduced bioavailable phosphorus concentrations and increased nitrate concentrations. Reduced phosphorus concentrations are primarily due to an 85 percent reduction in total phosphorus loading from the Detroit River (the Detroit River accounts for 93 percent of the inflow to Lake Erie) since 1968 (Hartig 1983). This reduction in phosphorus loading can be attributed primarily to phosphorus control measures in the basin (IJC 1981; Hartig and Horvath 1982; Hartig 1983). For example, approximately 40-50 percent of this reduction in total phosphorus loading from the Detroit River to the western basin alone can be explained by municipal phosphorus control measures at Detroit, Michigan (Hartig 1983). Since 1966, effluent total phosphorus concentration and loading from the Detroit Wastewater Treatment Plant (plant flow: 2,500,000 $m^3\ day^{-1}$) to the Detroit River have decreased over 90 percent. Increased nitrate concentrations in Pigeon Bay are probably the result of the combination of: 1) lower phosphorus concentrations which led to reduced algal biomass (Nicholls et al. 1980) and helped relieve exhaustion of nitrate; and 2) increased inputs of nitrogen from acid rain (Summers and Whelpdale 1976), agricultural runoff of fertilizer, and wastewater treatment plants. Nitrate

loadings from the Detroit River to the western basin have increased approximately two-fold since the late 1960's (IJC 1981).

The 85 percent reduction in total phosphorus loading from the Detroit River to the western basin in combination with the western basin's high flushing rate (Lake Erie's flushing rate: approximately 3 years) have resulted in lower phosphorus concentrations, a reduction in phytoplankton (Nicholls et al. 1980; IJC 1981), and contributed to the shift from Pigeon Bay's being primarily nitrogen limited in summer to being primarily phosphorus limited. Reductions in blue-green algae have also been reported in Pigeon Bay (Nicholls et al. 1980) which, in response to a nitrogen limited situation, increase in abundance because of their ability to fix molecular nitrogen (Schindler 1977). The manifestation of nitrogen limitation in the western basin is believed to have been a secondary effect of phosphorus enrichment that stimulated algal growth and produced conditions favorable for nitrogen fixing blue-green algae (Schelske et al. 1978). All this argues for a partial reversal of cultural eutrophication in the Pigeon Bay waters of the western basin. Similar findings have also been reported from Lake Washington following diversion of municipal sewage (Edmondson and Lehman 1981).

It must be remembered that this study was performed at one station in the Pigeon Bay waters of the western basin. Considerable physicochemical and biological heterogeneity

exists within the western basin (Schelske and Roth 1973; DiToro et al. 1975). For example, higher phosphorus and nitrogen concentrations have been found in the southern portion of the basin than in the northern portion (Gachter et al. 1974). The nearshore waters along the southern shore are heavily influenced by the nutrient-rich Maumee River and have greater diffuse source tributary phosphorus inputs than do the nearshore waters along the northern shore (PLUARG 1978). Andrews (1948) reported that the western basin is composed of two distinct water masses - one in the northern and one in the southern part of the basin. The movement and characteristics of the northern water mass are influenced chiefly by the discharge of the Detroit River, whereas the southern water mass is strongly influenced by the discharge of the Maumee River. Simons (1976) has reported that the largest portion of the Detroit River inflow to Lake Erie flows north of Pelee Island. Therefore, any chemical or biological improvements in the western basin resulting from reduced phosphorus loadings from the Detroit River would be manifested most in the waters off the north shore. The southern portion of the western basin is physicochemically different from the northern portion and may not respond in the same manner or as quickly.

3. Influence of Light and Temperature on Growth and Photosynthesis of *F. crotonensis*

3.1 Introduction

In recent years high densities of *Fragilaria crotonensis* Kitton have periodically occurred in the Pigeon Bay waters of western Lake Erie for reasons not fully understood (Nicholls et al. 1980). Light and temperature are of fundamental importance to seasonal phytoplankton dynamics. Phytoplankton have definite temperature optima and tolerance ranges and also exhibit species specific responses to light intensity (Stoermer and Ladewski 1976; Eppley 1972). The ecological effects of light and temperature on growth and photosynthesis of phytoplankton are inseparable because of interrelationships between metabolism and light saturation (Wetzel 1983). The purpose of this study was to quantitatiyely determine, under variable light and temperature regimes, rates of growth and photosynthesis of an axenic clone of *F. crotonensis* isolated from Lake Erie. Such information should provide new understanding of the environmental requirements of *F. crotonensis* and lead to a better understanding of its anomalous increased density in Pigeon Bay in recent years (Nicholls et al. 1980).

3.2 Materials and Methods

3.2.1 Culture Establishment and Maintenance

Using a sterile pipette technique (Guillard 1973) a single clone of *F. crotonensis* was isolated from Lake Erie

in freshwater "WC" media (Guillard and Lorenzen 1972). This unialgal culture was then purified into axenic culture using antibiotic treatment incorporating penicillin G, streptomycin sulfate, and chloramphenicol (Hoshaw and Rosowski 1973) (see Appendix 1). Subsamples from these cultures were periodically plated out on bacterial growth media to assure that they were axenic. All subculturing was performed using standard aseptic techniques. Axenic stock cultures of F. crotonensis were maintained within standard laboratory growth chambers in freshwater "WC" media at an irradiance of 150 $\mu E\ m^{-2}s^{-1}$ and a 14 hr:10 hr light:dark photoperiod. Separate stock cultures were maintained at 5, 11, 17, and 23°C, which reflects in situ temperatures encountered by F. crotonensis.

3.2.2 Photosynthesis

Photosynthesis experiments were initiated by aseptically removing a 20 ml subsample from a stock culture of F. crotonensis in log growth and exposing it to low level sonication (to break up long chains) for 20 seconds. One ml aliquots of this sonicated culture were then aseptically inoculated into 14 Erlenmeyer flasks containing 50 ml of "WC" media. The flasks were then placed in a growth chamber, two each at six different irradiances and two wrapped in aluminum foil for estimation of dark uptake. Plexiglass frames with neutral density filters were used to obtain the six irradiance levels which ranged from 18-280 $\mu E\ m^{-2}s^{-1}$. Irradiance levels were measured with a LI-COR

LI-193SB spherical quantum sensor. These culture flasks were preconditioned for 1 hour at the experimental irradiances, then inoculated with 5 μCi of carrier free $Na_2{}^{14}CO_3$ and incubated for 3 hours. Upon completion of the 3 hour incubation the samples were immediately filtered on to 0.80 μm Millipore filters (HA: 47 mm diameter), washed with 50 ml of filtered tap water (adjusted to pH 2), and placed in scintillation vials containing 10 ml of 2-methoxyethanol-toluene fluor. Photosynthetic carbon uptake was determined by radioassay using a liquid scintillation counter (Beckman LS 7500). Samples were counted to a minimum of 10,000 counts and quenching was corrected using the external standard method. Cell densities were estimated by counting three 1 ml subsamples of the stock culture preserved in Lugol's solution at the beginning of each experiment. Photosyntnetic rates were expressed as mg C 10^{-9} cells h^{-1}. Photosynthesis experiments were performed at 5, 11, 17, and 23°C.

3.2.3 Growth

For growth experiments, twelve 125 ml Erlenmeyer flasks containing 50 ml of "WC" media were inoculated aseptically with 1 ml of an axenic stock culture in log growth. Paired flasks were placed in the growth chamber at six different irradiance levels. Plexiglass frames with neutral density filters were used to achieve the six irradiance levels (24-201 μE $m^{-2}s^{-1}$). Cultures were grown for seven days, during which each flask was shaken daily and rotated at

random within the growth chamber. Cell densities were established by counting subsamples of the stock cultures preserved (in Lugol's Solution) at the beginning of the experiment and all samples preserved at the end of the experiment. A minimum of 150 chains of F. crotonensis were counted from at least two subsamples from each preserved sample. Growth rates (doublings day^{-1}) were calculated from the following formula:

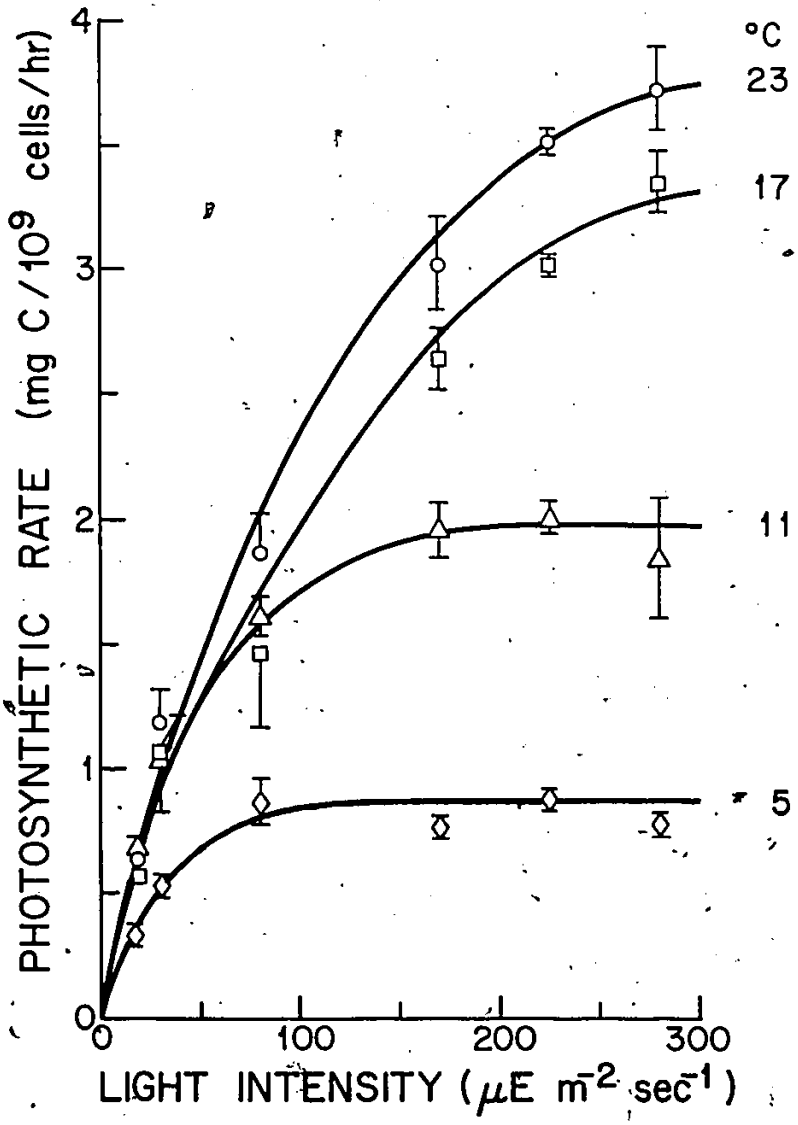
$$k = \frac{\ln N_2/N_1}{t_2 - t_1}$$

where k is the growth constant and N_1 and N_2 represent F. crotonensis densities at days 1 (t_1) and 8 (t_2). Growth experiments were performed at temperatures of 5, 11, 17, and 23°C.

3.3 Results

Curves showing the rates of F. crotonensis photosynthesis as a function of irradiance at four different temperatures (5, 11, 17, and 23°C) are presented in Figure 4. All curves follow rectangular hyperbolic functions. The initial slopes of the photosynthesis-irradiance (PI) curves adapted to 11, 17, and 23°C are similar. Yentsch (1980) and Jorgensen (1969) have suggested that this similarity in slope indicates that the concentrations of photosynthetic pigments in these cells are similar. When F. crotonensis was adapted to a temperature of 5°C the initial slope of the PI curve was reduced to 44 % of the slopes of the PI curves

Figure 4. Photosynthesis-light intensity curves of F. crotonensis as a function of temperature. Points on each curve represent mean and actual values.


PHOTOSYNTHETIC RATE (mg C / 10⁹ cells/hr)
LIGHT INTENSITY (μE m⁻² sec⁻¹)
°C
23
17
11
5
0
1
2
3
4
0
100
200
300

of F. crotonensis adapted to 11, 17, and 23°C. Microscopic examination showed no apparent change in the size of the cells adapted to 5°C. Consequently, since the rates of photosynthesis are presented per number of cells, the reduction in slope indicates that the concentration of photosynthetic pigments in cells adapted to 5°C was about half that of cells adapted to higher temperatures.

Light saturation (the horizontal portion of the PI curve) is limited by the rates of enzymatic processes, which are dependent on concentration of active enzymes and temperature (Soeder and Stengel 1974). At 17 and 23°C, under the light system used (maximum irradiance 280 $\mu E\ m^{-2}s^{-1}$), the maximum rates of photosynthesis (P_{max}) appear to be less than the absolute maximum obtainable by F. crotonensis although the curves suggest that these values are near the maximum (Figure 4). Therefore, for the purposes of this discussion these values will be referred to as P_{max} values. P_{max} values at 17 and 23°C were fairly similar (3.35 and 3.73 mg C 10^{-9} cells h^{-1}, respectively). P_{max} at 11°C was 1.96 mg C 10^{-9} cells h^{-1}, 52 % of P_{max} at 23°C. P_{max} at 5°C was 0.86 mg C 10^{-9} cells h^{-1}, 23 % of P_{max} at 23°C.

I_K (irradiance indicating onset of light saturation of photosynthesis) values were highest at 23°C (95 $\mu E\ m^{-2}s^{-1}$) and decreased with decreasing temperature (85 $\mu E\ m^{-2}s^{-1}$ at 17°C; 50 $\mu E\ m^{-2}s^{-1}$ at 11°C) to 45 $\mu E\ m^{-2}s^{-1}$ for cells adapted to the physiologically inferior environment at 5°C

(Figure 4). In comparing the I_K values of F. crotonensis found in this study (45-95 $\mu E\ m^{-2}s^{-1}$) with published I_K values of other phytoplankton (Table 6), it appears that the onset of light saturation of F. crotonensis occurs at relatively low irradiances.

The growth responses of F. crotonensis to increasing irradiance as a function of temperature (Figure 5) follow rectangular hyperbolic functions similar to the PI curves of F. crotonensis. Maximum growth rates (0.54 doublings day^{-1}) were found at 17 and 23°C. Maximum growth rates at 11 (0.35 doublings day^{-1}) and 5°C (0.17 doublings day^{-1}) were 68 and 31 %, respectively, of the maximum growth rates at 17 and 23°C. As was the case in the PI curves, the onset of light saturation in growth-irradiance curves (defined as the irradiance at which an extrapolation of the initial linear portion of the curve reaches maximum growth rate) was highest at 23°C (47 $\mu E\ m^{-2}s^{-1}$) and decreased with decreasing temperature to 22 $\mu E\ m^{-2}s^{-1}$ for cells grown in the physiologically inferior environment of 5°C.

Temperature coefficient (Q_{10}) values for growth of F. crotonensis were 1.4 (calculated from maximum growth rates at 11 and 23°C) and 1.8 (calculated from maximum growth rates at 5 and 23°C). Field sampling in Pigeon Bay (Section 2) has shown that at cold temperatures F. crotonensis can achieve population densities as high as 25 % of maximum summer density (Figure 6). Population densities in April were 230,000 cells L^{-1} at a water temperature of

Table 6. Published I_K values for selected algae.

Alga or group	I_k ($\mu E\ m^{-2} s^{-1}$)	Reference
Olisthodiscus luteus	90	Thomas (1980)
chlorophycean algae	100	Ryther (1956)
Nannochloris atomus	82 @ 5°C; 119 @ 10°C; 155 @ 20°C	Yentsch and Lee (1966)
Scenedesmus obliquus	132-198	Jorgensen (1969)
Chlorella pyrenoidosa	79-330	"
Chlorella vulgaris	53-171	"
Ankistrodesmus falcatus	92-224	"
Chlamydomonas moewusii	119-185	"
Monodus subterraneus	79	"
Synechococcus elongatus	66-132	"
Skeletonema costatum	119-185	"
Cyclotella meneghiniana	119-171	"
Nitzschia palea	105-171	"
Nitzschia closterium	119-211	"
Scenedesmus quadricauda	119-211	"
Isochrysis galbana	300	Dunstan (1973)
Chlorella huxleyi	281	"
Cyclotella nana	277	"
Amphidinium carteri	286	"
Prorocentrum micans	291	"
Anabaena variabilis	42 @ 10°C; 99 @ 15°C; 150 @ 20°C; 235 @ 25°C; 350 @ 30°C; 435 @ 35°C; 564 @ 40°C	Collins and Boylen (1982)
temperate diatoms	226	Quasim et al. (1972)
dinoflagellates	516	"
tropical phytoplankton	645-1935	"

Figure 5. Growth-light intensity curves of *F. crotonensis* as a function of temperature. Points on each curve represent the mean and actual values.

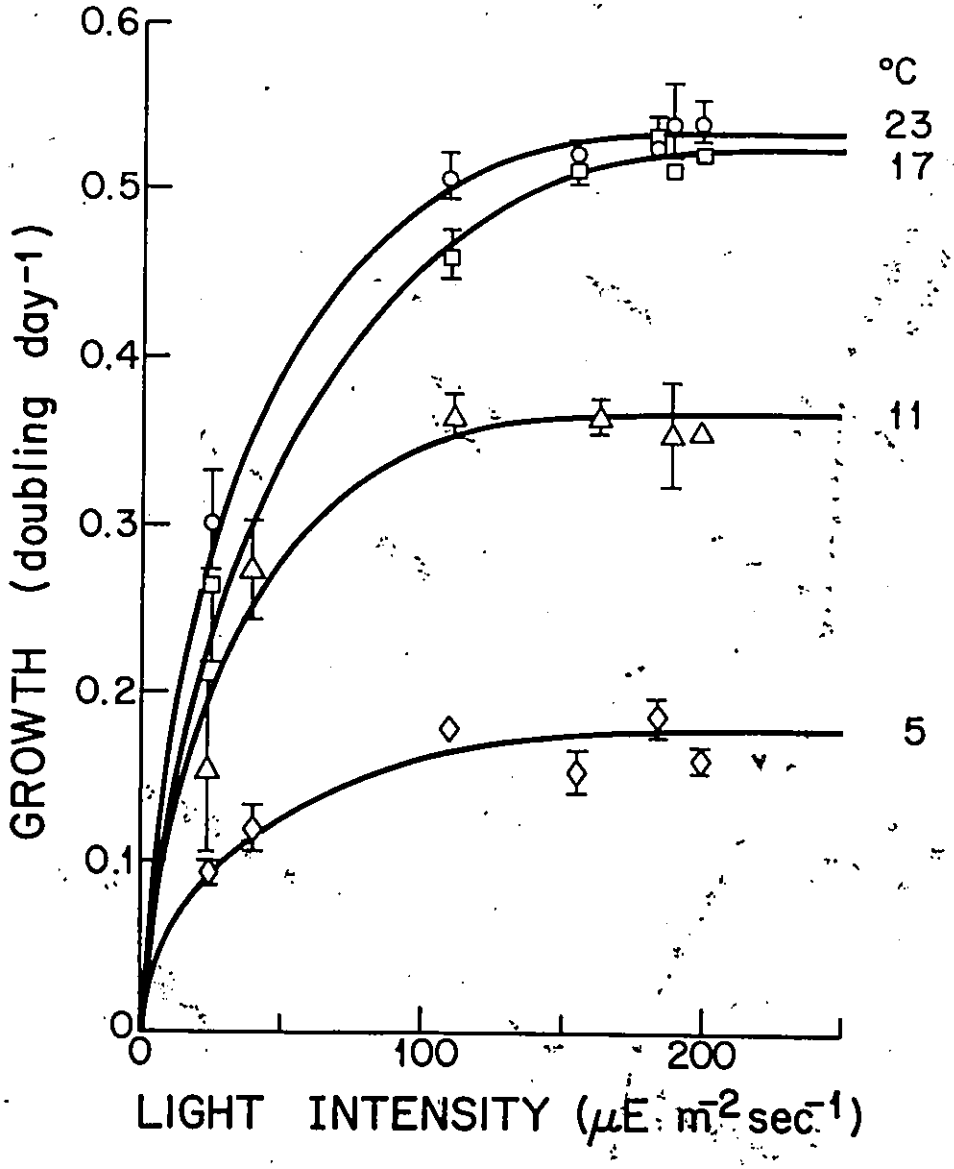
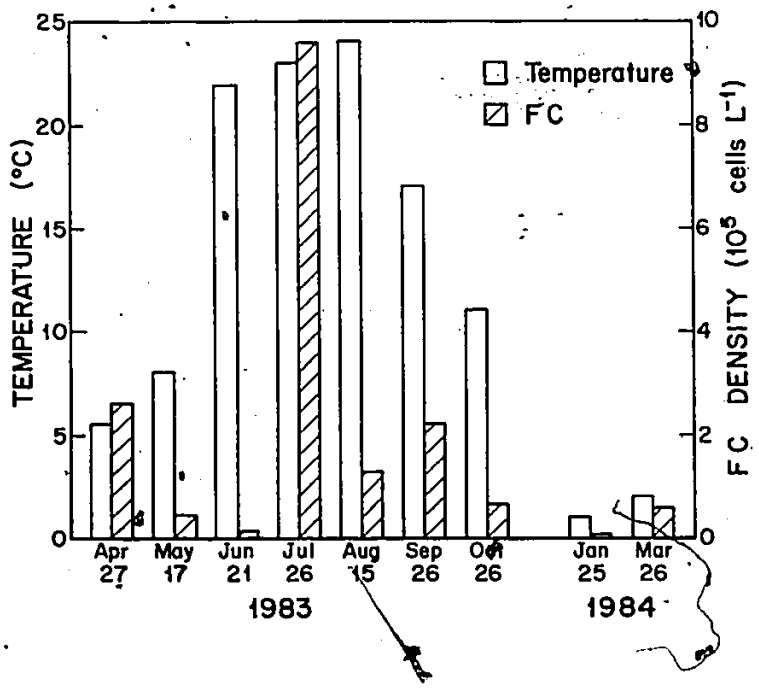

0.6
0.5
0.4
0.3
0.2
0.1
0
GROWTH (doubling day-1)
°C
23
17
11
5
0
100
200
LIGHT INTENSITY (μE m-2 sec-1)

Figure 6. Monthly densities of F. crotonensis and water temperatures from a station (42°02'53"N; 82°41'00"W) in the Pigeon Bay waters of western Lake Erie, April, 1983 to March, 1984. FC = F. crotonensis.


Temperature
FC
TEMPERATURE (°C)
FC DENSITY (10^5 cells L^{-1})
0
5
10
15
20
25
0
2
4
6
8
10
Apr 27
May 17
Jun 21
Jul 26
Aug 15
Sep 26
Oct 26
Jan 25
Mar 26
1983
1984

5°C, compared to 950,000 cells L^{-1} at 23°C in July. High population densities of F. crotonensis in Pigeon Bay at cold water temperatures is consistent with previous findings of Nicholls et al. (1980).

3.4 Discussion

Growth and photosynthesis of phytoplankton are dependent on the complex interaction of light, temperature, and nutrients (Steemann-Nielsen and Jorgensen 1968). With all nutrients in excess, growth and photosynthesis of phytoplankton in batch culture are strongly affected by the interaction between light and temperature (Morgan and Kalff 1979).

The shape of photosynthesis-irradiance (PI) curves provides important information about adaptation (physiological adjustment to surrounding conditions) of algae. Even though the slopes of the PI curves of F. crotonensis at 11, 17, and 23°C are the same (indicating comparable photosynthetic pigment concentrations), maximum rates of photosynthesis (P_{max}) are different. P_{max} values of cells grown at 17 and 23°C were relatively high and fairly comparable. At 11°C, P_{max} was 52 % of P_{max} at 23°C. Fragilaria crotonensis adapted to 11°C by varying the light saturated rate of photosynthesis (i.e. by varying the maximum rates of enzymatic processes). The 5°C PI curve, which exhibits a shift in both slope and P_{max}, provides evidence that F. crotonensis at this low temperature was unable to maintain the higher concentrations of

photosynthetic pigments or enzymes needed to counteract the effect of low temperature. However, the resulting concentration of photosynthetic pigments and enzymes was sufficient to maintain photosynthetic rates at 23 % of P_{max} achieved at 23°C. These results are similar to other studies of phytoplankton adaptation to temperature which indicate a low P_{max} at low temperatures (Yenstch and Lee 1966; Steemann-Nielsen and Jorgensen 1968).

The irradiance indicating onset of light saturation of photosynthesis (I_K) is often used as an indication of the light adapted condition of phytoplankton (Jones 1978). In this study I_K values decreased with decreasing temperature. Because the initial slopes of the 11, 17, and 23°C curves are the same, I_K is solely determined by P_{max}, which is known to be strongly temperature dependent (Soeder and Stengel 1974). At 5°C, the I_K for F. crotonensis appears to be the result of a shift in both the initial slope of the curve (which reflects pigment concentrations) and P_{max}. The decrease in I_K values with decreasing temperature shows that in a suboptimal environment F. crotonensis reaches a lower photosynthetic maximum at lower irradiances. This is considered characteristic of many diatoms (Jones 1978). Both lowered P_{max} and lowered I_K show the adaptation of F. crotonensis to a physiologically inferior environment. Further, because these I_K values are within a fairly narrow range (45-95 $\mu E\ m^{-2}s^{-1}$), this suggests that F. crotonensis may be adapted to sudden changes in irradiance (Jorgensen

and Steemann-Nielsen 1965). However, it must be remembered that these photosynthesis experiments were performed in the laboratory and that phytoplankton adaptation to variation in irradiance in the natural environment may take several days (Harris 1978).

The growth-irradiance curves of F. crotonensis are similar in shape to the PI curves and further demonstrate adaptation of F. crotonensis to varying light and temperature. Since growth of algae depends on the rate of metabolic processes (which are temperature dependent), one would expect some temperature variation of specific growth rate if conditions were otherwise suitable for growth (i.e. light and nutrients are not limiting). The onset of light saturation in these growth-irradiance curves decreased with decreasing temperature. This shows that at suboptimal temperatures growth of F. crotonensis reaches light saturation at lower irradiances and shows adaptation of F. crotonensis to a physiologically inferior environment. As was the case in the 5°C PI curve, the 5°C growth-irradiance curve demonstrates a physiological adjustment in concentrations of photosynthetic pigments and active enzymes to maximize growth at low temperature.

In this study maximum growth rates of F. crotonensis were 0.53 doublings day^{-1} at 23 and 17°C. These maximum growth rates of the Lake Erie clone of F. crotonensis appear to be slightly less than the maximum growth rates of other clones of F. crotonensis. Based on batch growth experiments

performed at 20°C, 100 $\mu E\ m^{-2}s^{-1}$ illumination, and a 14 hr:10 hr light:dark cycle Tilman (1981) found maximum growth rates of a clone of F. crotonensis isolated from Lake Michigan of 0.62 doublings day^{-1} in silica limited experiments and 0.80 doublings day^{-1} in phosphate limited experiments. Based on growth experiments performed at 14°C, approximately 50 $\mu E\ m^{-2}s^{-1}$, and continuous light Tompkins and Blinn (1976) found a maximum growth rate of 0.72 doublings day^{-1} for a clone of F. crotonensis isolated from Lake Powell, Arizona. These experiments performed in continuous light, however, cannot be considered representative because algae normally grow on a light:dark cycle.

In general, it is thought that diatoms have an extended range of temperature for good growth into lower temperatures (Wetzel 1983). If 31 (at 5°C) and 68 % (at 11°C) of maximum growth (at 17 and 23°C) can be considered good growth, then F. crotonensis can grow well at lower temperatures. Even over a broad range of species and temperature optima it appears that van't Hoff's Q_{10} Rule (i.e. biological processes increase nearly two-fold with each 10°C rise in temperature) holds true for phytoplankton growth (Goldman and Carpenter 1974; Talling 1955; Eppley 1972; Welch 1980). Assuming the Q_{10} Rule holds true for phytoplankton growth, then F. crotonensis's Q_{10} values of 1.4 (based on 11-23°C) and 1.8 (based on 5-23°C) suggest that it is adapted to seasonal or other fluctuations in temperature (i.e. it is

eurythermal). Field data from Pigeon Bay have shown that F. crotonensis can withstand 20°C temperature variation and that at 2.0 and 5.5°C it can achieve population densities of 6 and 27 %, respectively, of maximum summer density at 23.5°C. Diatoms that can live in 20°C or more variation in temperature are classified eu-eurytherms (Patrick 1977). Stoermer and Ladewski (1976) indicated that F. crotonensis is either quite eurythermal or is represented by more than one physiological race in Lake Michigan. Maximum population densities were found at water temperatures near 15°C, but there was a strong secondary peak in abundance near 7°C. From laboratory experiments Rodhe (1948) determined that F. crotonensis's temperature optimum to be between 12 and 15°C, yet its maximum in nature is from 15-29°C. Fragilaria crotonensis from Bodensee-Obersee has a range of temperature permitting growth from less than 15°C to greater than 30°C with an optimum temperature of 26°C (Muller 1972). The ubiquitous distribution and common occurrence of F. crotonensis (Hutchinson 1967) also attests to its wide temperature tolerance range.

The onset of light saturation of both photosynthesis (I_K: 45-95 $\mu E\ m^{-2}s^{-1}$) and growth (<50 $\mu E\ m^{-2}s^{-1}$) of F. crotonensis occurred at fairly low irradiances. This suggests that it is adapted to a low light environment. In Lake Michigan, Fahnenstiel et al. (1984) have investigated nutrient-light interactions via semicontinuous culture experiments on natural phytoplankton assemblages collected

from the "deep" chlorophyll layer (30 m). At steady state, F. crotonensis became the dominant alga at irradiances of 8-26 $\mu E\ m^{-2}s^{-1}$ (G.L. Fahnenstiel, Great Lakes Environmental Research Laboratory, 1984). These results provide additional evidence that F. crotonensis can be adapted to a low light environment. Fragilaria crotonensis may also be adapted to sudden changes in irradiance based on the narrow range of I_k values (Jorgensen and Steemann-Nielsen 1965). In the productive waters of western Lake Erie where the depth of the photic zone is often less than the depth of the surface mixed layer, F. crotonensis's apparent adaptation to a low and variable light environment may give it a competitive advantage over other phytoplankton at certain times.

Even though F. crotonensis appears to be adapted to a low light environment, it is probable that it is at least periodically light limited in the turbid (Paul et al. 1982) and productive (Nicholls et al. 1980) waters of the western basin. Monaco et al. (1982) have reported that light attenuates to 50 $\mu E\ m^{-2}s^{-1}$ in the western basin at depths varying from less than 2 m to over 7 m. Lean and Nalewajko (1979) have suggested that phytoplankton growth was light limited at a non-stratified station in western Lake Erie due to high turbidity resulting in a 1 % light level of 2.2 m. Further, because of the shallow and frequently turbulent nature of the western basin, F. crotonensis may infrequently be light limited if most of the time it receives sufficient

light from being alternately carried into and out of the photic zone through vertical mixing. Little is known about the effects of periodic exposure to the photic zone on growth of phytoplankton. Walsby and Reynolds (1980) suggest that vertical mixing in lakes generally favors the dominance of diatoms because they are adapted physiologically to contend with rapid fluctuations of irradiance. Further experimental work with F. crotonensis is warranted using photoperiod and fluctuating irradiance as independent variables.

4. Light and Nutrient Limitation of *F. crotonensis*

4.1 Introduction

In recent years, high densities of *Fragilaria crotonensis* have occurred periodically in the Pigeon Bay waters of western Lake Erie for reasons not fully understood (Nicholls et al. 1980). Such "plankton pulses" are believed to be caused by one or more physical, chemical, or biological factors. In the western basin of Lake Erie turbidity influences the quality and quantity of light available at various depths, which in turn can influence the time, duration, and size of "plankton pulses" (Chandler 1940). Resource competition theory predicts that under steady state conditions the relative abundance of different species within the phytoplankton community is the result of varying abilities to acquire and utilize nutrients (Titman 1976; Tilman et al. 1982). In addition, combined effects of light and nutrients on growth of phytoplankton have been demonstrated to be greater than the sum of individual effects (Rhee and Gotham 1981). The purpose of this study was to investigate the effects of light and nutrients on growth of *F. crotonensis* from Pigeon Bay. Effects of light were investigated by analysis of Pigeon Bay field data (Pigeon Bay Monitoring) and by performing experiments which incorporate light as an independent variable (1984 Resource Competition Experiments). Effects of nutrients and the combined effects of light and nutrients were investigated by performing experiments which incorporate them as

independent variables (1983 Nutrient Enrichment Experiments and 1984 Resource Competition Experiments).

4.2 Methods and Materials

4.2.1 Union Water Intake (UWI) Monitoring

As stated in Section 2, Ontario Ministry of the Environment (OME) has performed weekly monitoring of Pigeon Bay via the UWI to help evaluate long-term trends in water quality. Weekly Fragilaria spp. biomass measurements have been made since 1967 and are published in Nicholls et al. (1980). In addition, UWI staff have performed daily monitoring of turbidity as part of their treatment process (D. Jones, UWI, 1984). Influent water samples are obtained and turbidity immediately measured on a Hach Turbidimeter in formazin turbidity units (FTU). This Hach Turbidimeter technique has been consistently used since 1977.

4.2.2 1983 Nutrient Enrichment Experiments

As part of the monthly sampling program to identify nutrients limiting chlorophyll a production from April-October, 1983 (Section 2), phytoplankton samples were preserved in Lugol's solution both before and after each experiment. Fragilaria crotonensis were enumerated in selected samples (those most likely to exhibit a growth response based on increased chlorophyll concentration; Section 2) from each of these experiments using an inverted microscope (Utermöhl 1958). Mean daily growth rates over the incubation period were calculated for each treatment using the same growth equation presented in Section 2.

One-way analysis of variance procedures were used to determine if nutrient enrichment did significantly affect F. crotonensis growth rate. Duncan's multiple range tests were used to determine which treatments resulted in significantly different growth rates (Zar 1974).

4.2.3 1984 Resource Competition Experiments

On 25 January and 26 March 1984 water samples containing natural phytoplankton were collected from 1.5 m at the Pigeon Bay station. Samples were transported to the laboratory (within two hours) in the dark and at ambient water temperature.

Immediately upon arrival in the laboratory, the lake water sample was thoroughly mixed and one liter filtered through a 47 mm Millipore filter (0.45 µm pore size). The filtrate was then analyzed for soluble reactive phosphorus, total soluble phosphorus, nitrate, and reactive silicate using standard methods (Section 2). Three 50 ml subsamples of lake water were also preserved in Lugol's solution for phytoplankton identification and enumeration using an inverted microscope (Utermöhl 1958).

For each experiment, the lake water sample was thoroughly mixed and 1500 ml dispensed into eight 2-L polycarbonate bottles. These eight bottles were divided into two 4-bottle sets, each set including a control, a phosphorus enrichment (+P: 20 µg L^{-1}), a silica enrichment (+Si: 500 µg L^{-1}), and a phosphorus and silica enrichment (+P&Si: 20 and 500 µg L^{-1}, respectively). Phosphorus and

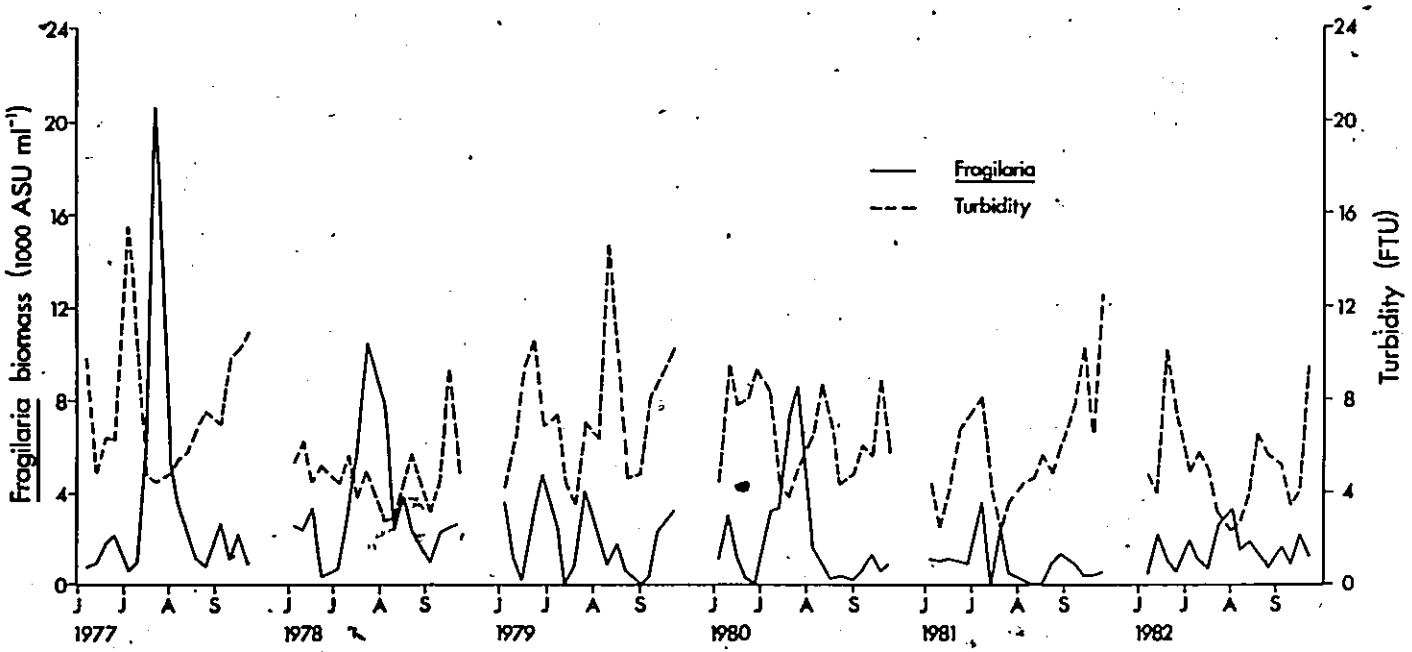
silica concentrations were brought up to desired levels by dispensing aliquots of stock solutions. During the experiments phosphorus and silica levels were maintained at 20 $\mu g\ L^{-1}$ and 500 $\mu g\ L^{-1}$, respectively, by measuring concentrations every 5 days and enriching if necessary. Control treatments received aliquots of distilled water comparable in volume to the phosphorus and silica enrichments. One set of bottles was placed in a growth chamber at 60 $\mu E\ m^{-2}s^{-1}$ and the other set placed in another growth chamber at 120 $\mu E\ m^{-2}s^{-1}$. Irradiances were measured with a LI-COR LI-193SB spherical quantum sensor. Light:dark cycle and growth chamber temperature were 10 hr:14 hr and 3°C, respectively, for the January experiment and 12 hr:12 hr and 5°C, respectively, for the March experiment. Bottles were incubated for 30 days during the January experiment and 20 days during the March experiment, during which each bottle was shaken daily and repositioned at random. Phytoplankton were subsampled (50 ml) from each bottle every 10 days during the January experiment and every 5 days during the March experiment and preserved in Lugol's Solution for subsequent identification and enumeration.

4.3 Results

4.3.1 Union Water Intake Monitoring

Weekly *Fragilaria* spp. biomass data (primarily *F. crotonensis*) from June-September, 1977-1982 are presented in Figure 7. The June-September period was evaluated because water temperatures during these months were generally

Figure 7. Weekly turbidity (7-day average) and Fragilaria spp. biomass data collected from Pigeon Bay via the Union Water Intake, June-September, 1977-1982. Fragilaria biomass data were collected by OME. Turbidity data were collected by the Union Water Intake, Kingsville, ON.


Fragilaria biomass (1000 ASU ml⁻¹)
Turbidity (FTU)
Fragilaria
Turbidity
0
4
8
12
16
20
24
J J A S
1977
1978
1979
1980
1981
1982

between 17 and 24°C, which reflects the temperature range within which axenic F. crotonensis exhibited highest growth and photosynthesis rates (see Section 3). The mean turbidity value for the 7-day period preceding each weekly Fragilaria biomass measurement (e.g. if Fragilaria biomass was measured on June 9, a mean turbidity value for June 3-9 was calculated) is also presented in Figure 7 as an indication of light conditions leading up to the Fragilaria biomass in Pigeon Bay. Both Fragilaria biomass (range: 0-20,613 Areal Standard Units or ASU ml^{-1}) and turbidity (range of 7-day average: 2.3-15.4 FTU) are quite variable, reflecting the dynamic and rapidly fluctuating nature of Pigeon Bay. Based on linear regression analysis, no significant ($P<0.01$) linear regression exists between turbidity and Fragilaria biomass (Figure 8). Even though no significant linear regression exists between turbidity and Fragilaria biomass during summer in Pigeon Bay, it is of interest to note that the eight phytoplankton samples (all collected during July) which exhibited Fragilaria "pulses" were collected when turbidity during the preceding week was $\leq$ 5 FTU (mean: 4.29 FTU; 95% confidence interval: 0.67 FTU). This observation of low turbidity preceding each Fragilaria "pulse" is further supported by the large F. crotonensis "pulse" (950,000 cells L^{-1}) observed on July 26, 1983 when turbidity during the preceding week averaged 2.86 FTU (Section 3). Figure 9 presents the significant linear regression between mean weekly wind speed and turbidity in

Figure 8. Linear regression of Fragilaria spp. biomass on turbidity, June-September, 1977-1982. Analysis of variance showed that Fragilaria spp. biomass is not dependent upon turbidity ($F=4.15$; $P>0.01$).

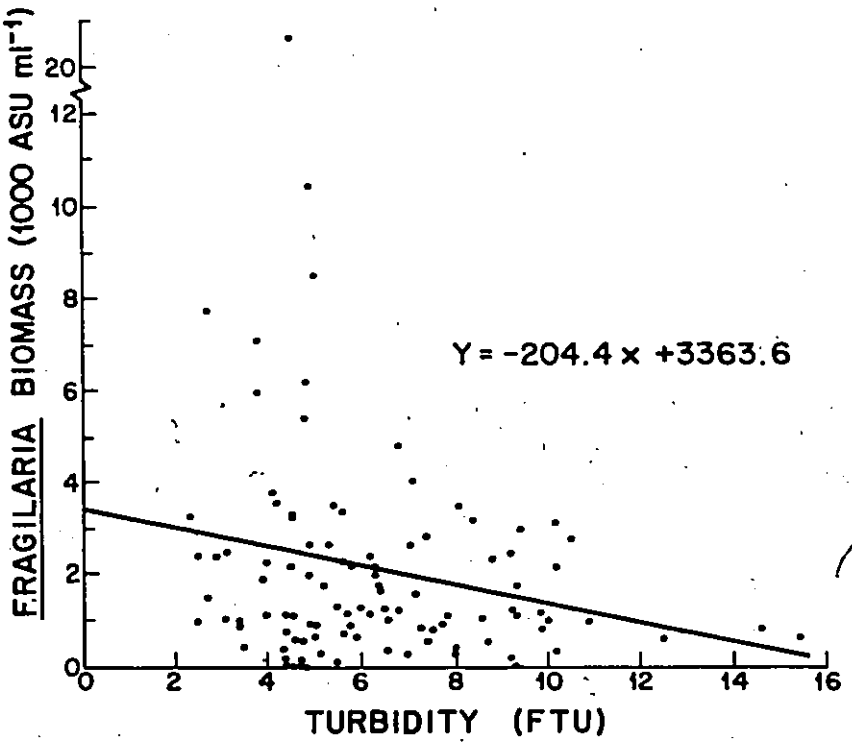
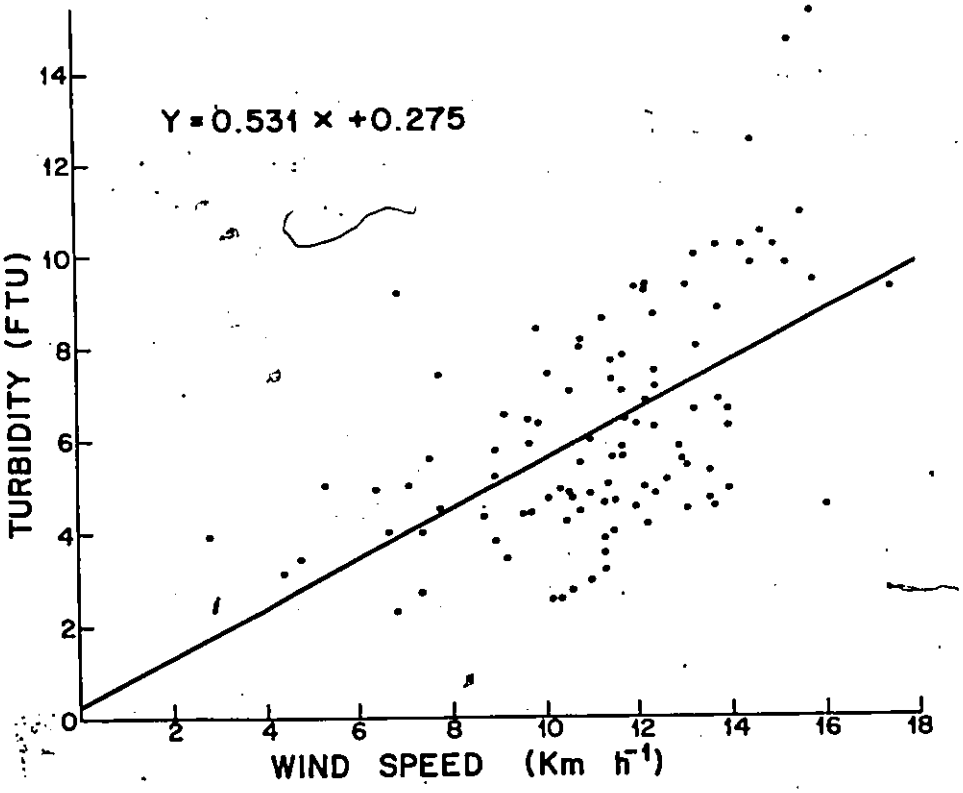

F.RAGILARIA BIOMASS (1000 ASU ml⁻¹)
20
12
10
8
6
4
2
0
Y = -204.4 x +3363.6
0
2
4
6
8
10
12
14
16
TURBIDITY (FTU)

Figure 9. Linear regression of turbidity on wind speed, June-September, 1977-1982. Analysis of variance showed that turbidity is dependent upon wind speed in Pigeon Bay ($F=45.48$; $P<0.01$). Wind speed data were recorded at the Pt. Pelee Weather Station (Pt. Pelee National Park, Ontario).


Y = 0.531 × +0.275
TURBIDITY (FTU)
WIND SPEED (Km h⁻¹)
0
2
4
6
8
10
12
14
16
18

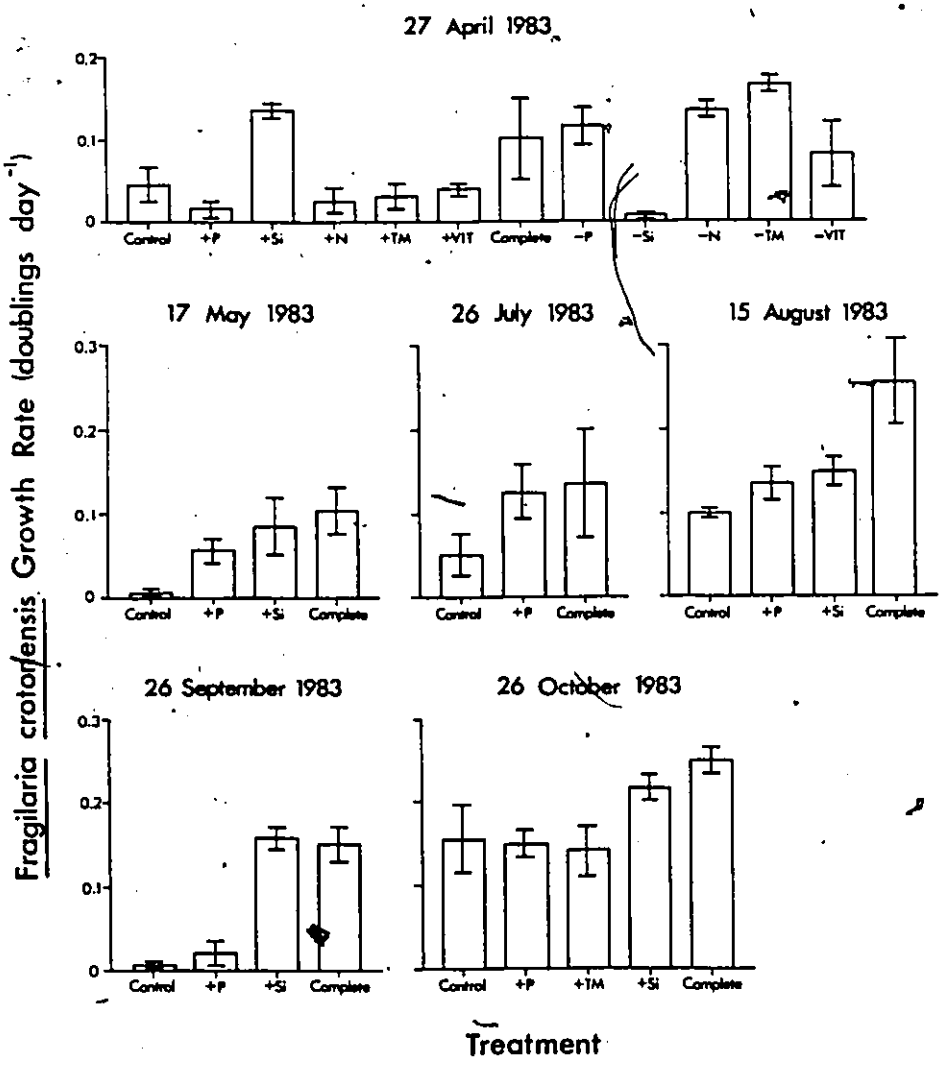
Pigeon Bay during the same summer period (June-September, 1977-1982).

4.3.2 1983 Nutrient Enrichment Experiments

Figure 10 presents F. crotonensis growth rate data from selected treatments employed in nutrient enrichment experiments performed in April, May, and July-October. Growth rate data were not calculated for June because of the presence of attached algal growth on the sides of all flasks enriched with phosphorus (see Section 2). The July, August, and September data are probably not truly representative of maximum F. crotonensis growth rates over 9 days because chlorophyll a concentrations in the experimental treatments decreased between days 6 and 9 which indicated the phytoplankton had become nutrient limited (Section 2). This nutrient limitation would limit further growth. Despite this fact, these data are presented as an indication of potential nutrient limitation of F. crotonensis growth.

One-way analysis of variance procedures performed on these data showed that nutrient enrichment significantly affected F. crotonensis growth during April, August, September, and October (α level: 0.05). In April and September, when silica concentrations were less than 170 μg L^{-1} (Section 2; Table 3), F. crotonensis growth rates ranged from 0.08-0.15 doublings day^{-1} in silica enrichments, compared to less than or equal to 0.04 doublings day^{-1} in unenriched controls (Figure 10). In October, when silica concentration was less than 230 μg L^{-1} (Section 2; Table 3),

Figure 10. *Fragilaria crotonensis* growth rates from selected treatments of nutrient enrichment experiments, April-October, 1983. Data are means of triplicate experiments, error bars are plus and minus one standard error.


27 April 1983
17 May 1983
26 July 1983
15 August 1983
26 September 1983
26 October 1983
Control
+P
+Si
+N
+TM
+VIT
Complete
-P
-Si
-N
-TM
-VIT
Fragilaria crotonensis Growth Rate (doublings day⁻¹)
Treatment

mean F. crotonensis growth rates were greater than 0.21 doublings day^{-1} in silica enrichments and less than 0.16 in all treatments not enriched with silica (Figure 10). Duncan's multiple range test groupings indicate that during these months silica was probably the nutrient most limiting F. crotonensis growth (Table 7). Even though no phytoplankton data were obtained from the June experiment because of attached algal growth, low silica concentration (75 µg L^{-1}) was potentially limiting F. crotonensis growth. Union Water Intake data have shown that silica depletion occurs during spring, early summer, and fall in Pigeon Bay (Figure 11).

Analysis of August data showed that the complete treatments (which included P, Si, N, vitamins, and trace metals) resulted in a mean F. crotonensis growth rate of 0.26 doublings day^{-1} which was significantly higher ($P<0.05$) than F. crotonensis growth in the control, +P, and +Si treatments (mean growth rates: < 0.15 doublings day^{-1}; Table 7). However, because there were no significant differences between the growth rates of the +Si, +P, and control treatments, the major nutrient limiting F. crotonensis growth cannot be identified from these August data.

Analysis of variance showed that nutrient enrichment did not significantly affect F. crotonensis growth in May and July (α level: 0.05) when growth rates were less than 0.14 doublings day^{-1}. It is not clear why F. crotonensis

Table 7. Mean F. crotonensis growth rates (doublings day^{-1}) observed in selected nutrient enrichment experiments performed during April, September, and October, 1983 with corresponding Duncan's multiple range test groupings ($P<0.05$). Means with the same letter are not significantly different.

APRIL

Treatment	Mean Growth Rate	Duncan's Multiple Range Test
-TM	0.165	A
+SI	0.135	A B
-N	0.135	A B
-P	0.117	A B
Complete	0.097	A B C
-VIT	0.079	B C D
Control	0.045	C D E
+VIT	0.040	C D E
+TM	0.030	C D E
+N	0.023	D E
+P	0.018	D E
-SI	0.004	E

AUGUST

Treatment	Mean Growth Rate	Duncan's Multiple Range Test
Complete	0.257	A
+SI	0.146	B
+P	0.132	B
Control	0.100	B

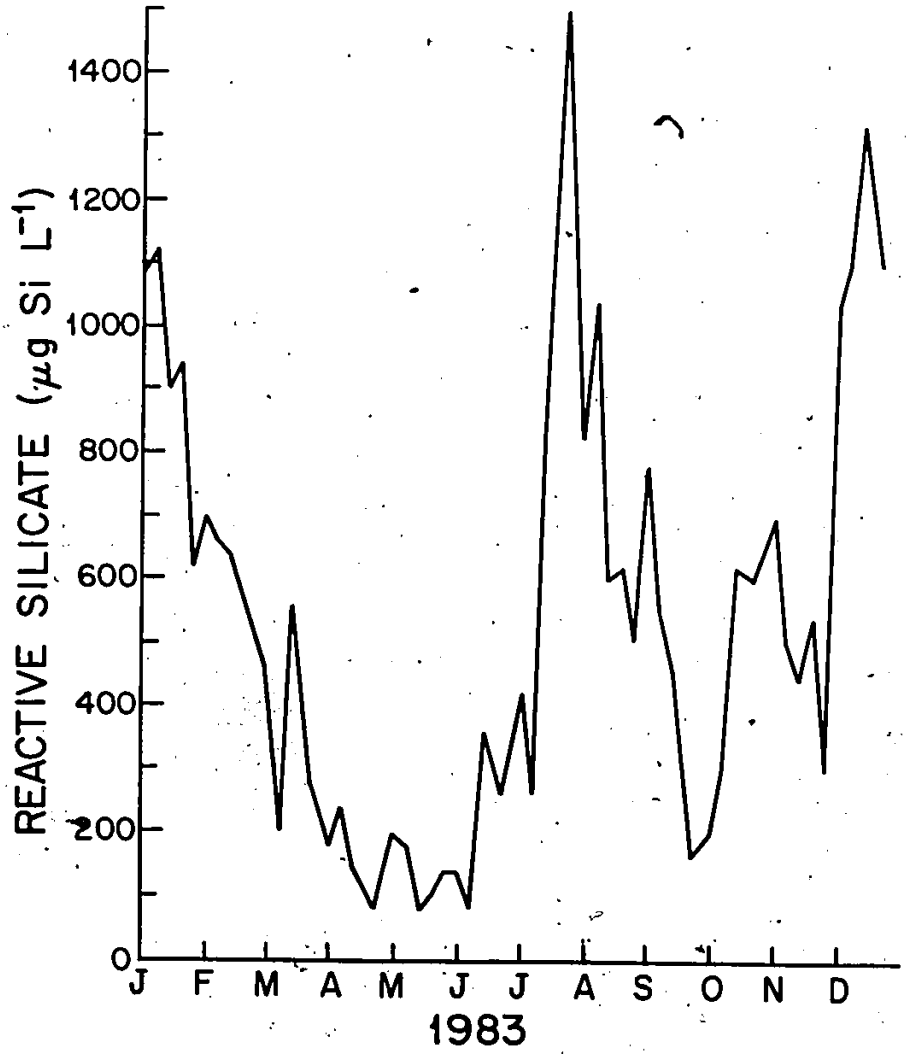
SEPTEMBER

Treatment	Mean Growth Rate	Duncan's Multiple Range Test
+SI	0.158	A
Complete	0.150	A
Control	0.024	B
+P	0.022	B

OCTOBER

Treatment	Mean Growth Rate	Duncan's Multiple Range Test
Complete	0.247	A
+SI	0.214	A B
Control	0.156	B
+P	0.148	B
+TM	0.141	B

Figure 11. Seasonal periodicity of reactive silicate in water samples from Pigeon Bay collected at the Union Water Intake, 1983. Data were collected by Ontario Ministry of the Environment (G.J. Hopkins, OME, 1984).


REACTIVE SILICATE (μg Si L^{-1})
1400
1200
1000
800
600
400
200
0
J F M A M J J A S O N D
1983

did not respond significantly to nutrient enrichment in May. In July, the conclusion that phosphorus enrichment did not affect F. crotonensis growth (Figure 10) may be misleading because (as noted earlier) by day 6 of the experiment the phytoplankton had used up most of the phosphorus. This resulted in a decrease in chlorophyll a concentration between day 6 and 9 (Section 2) and would restrict growth of F. crotonensis.

4.3.3 Resource Competition Experiments

Total phytoplankton density was at a seasonal minimum of approximately 700,000 cells L^{-1} at the outset of the January experiment (Figure 12). Green algae dominated, accounting for 60 percent of the community. Diatoms, the second major group, accounted for 27 percent of the community. Fragilaria crotonensis accounted for less than one percent of the community (700 cells L^{-1}).

Only phosphorus enrichment treatments were employed in the January experiment because silica was always present in concentrations greater than 800 µg L^{-1} (Table 8). Throughout the experiment concentrations of soluble reactive phosphorus in control and phosphorus enrichment treatments were approximately 6 and 20 µg L^{-1}, respectively (Table 9). At the outset of the experiment nitrate was 260.3 µg L^{-1} and total soluble phosphorus was 61.3 µg L^{-1}.

Increased light intensity, phosphorus enrichment, or increased light intensity plus phosphorus enrichment did not enhance growth of F. crotonensis or total phytoplankton

Figure 12. Seasonal periodicity of phytoplankton density at a station in the Pigeon Bay waters of western Lake Erie, 1983-1984.

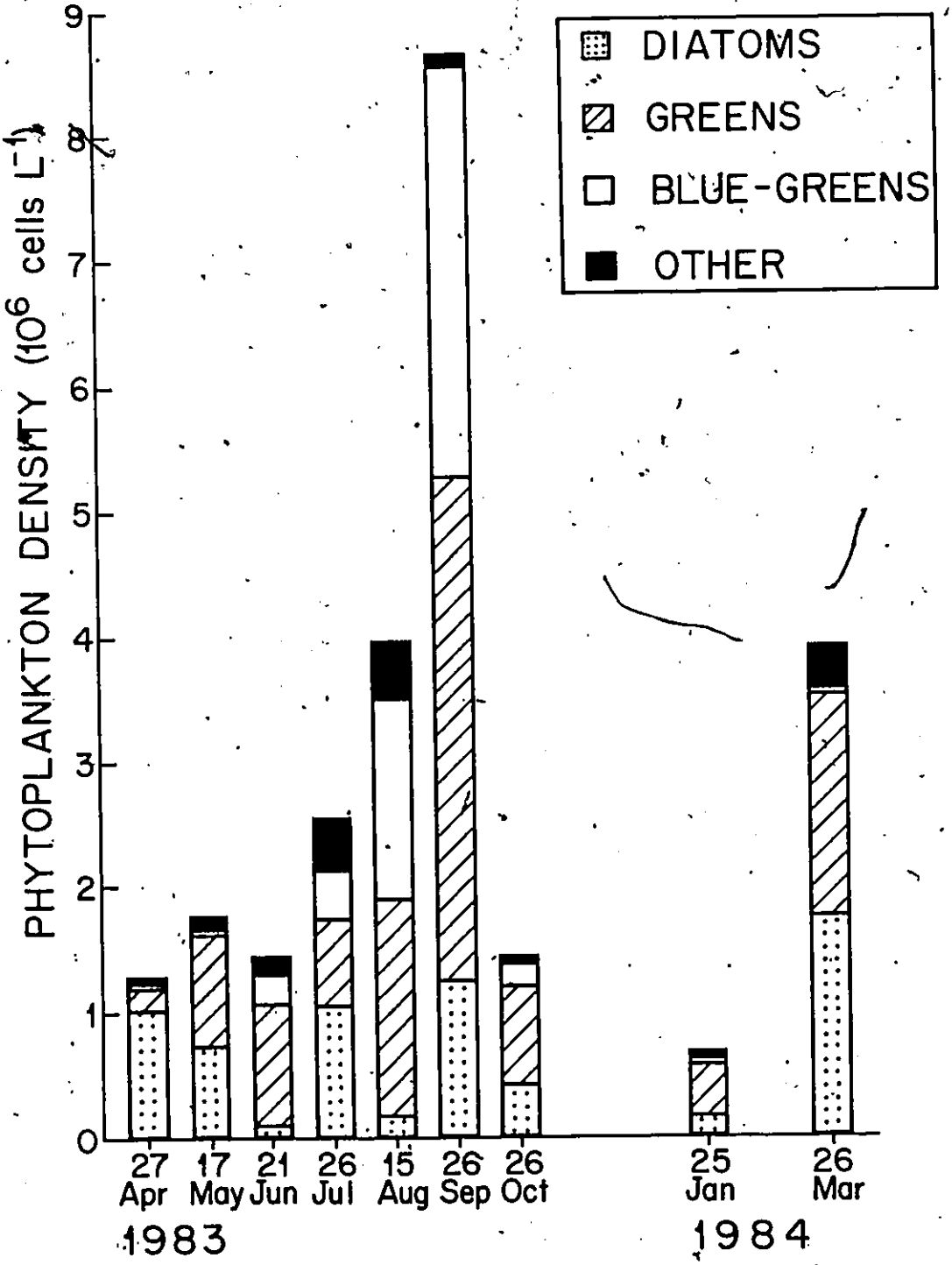

DIATOMS
GREENS
BLUE-GREENS
OTHER
PHYTOPLANKTON DENSITY (10^6 cells L^{-1})
0
1
2
3
4
5
6
7
8
9
27 Apr
17 May
21 Jun
26 Jul
15 Aug
26 Sep
26 Oct
25 Jan
26 Mar
1983
1984

Table 8. Reactive silicate concentrations ($\mu g\ L^{-1}$) in the different treatment bottles throughout the 30-day experiment, January, 1984. ND=no data.

	60 μE m^{-2} s^{-1}		120 μE m^{-2} s^{-1}	
Day	Control	P-enrichment	Control	P-enrichment
0	912.4	912.4	912.4	912.4
5	ND	918.0	ND	960.9
10	ND	906.6	ND	907.9
15	889.6	889.6	891.5	888.9
20	ND	866.2	ND	847.2
25	ND	ND	ND	ND
30	854.8	840.7	848.1	849.9

Table 9. Soluble reactive phosphorus concentrations ($\mu g\ L^{-1}$) in the different treatment bottles throughout the 30-day experiment, January, 1984. ND=no data.

	60 μE m^{-2} s^{-1}		120 μE m^{-2} s^{-1}	
Day	Control	P-enrichment	Control	P-enrichment
0	6.2	19.5	6.2	19.5
5	ND	18.8	ND	19.7
10	ND	17.8	ND	21.5
15	5.8	19.2	7.2	19.2
20	5.2	22.8	ND	25.6
25	ND	ND	ND	ND
30	5.9	20.8	5.8	22.9

assemblages during the January experiment (Figure 13). Density of F. crotonensis at the beginning of the experiment (day 0) was 700 cells L^{-1}. After day 0, F. crotonensis was only found in a phosphorus enrichment treatment at 120 μE $m^{-2}s^{-1}$ on day 10 (5,200 cells L^{-1}), a phosphorus enrichment treatment at 60 μE $m^{-2}s^{-1}$ on day 20 (2,400 cells L^{-1}), and a phosphorus enrichment treatment at 60 μE $m^{-2}s^{-1}$ on day 32 (800 cells L^{-1}).

The March experiment was initiated 4 days after the ice moved off Pigeon Bay. Total phytoplankton density appeared to be at a spring maximum of approximately 3,900,000 cells L^{-1} at the outset of the experiment (Figure 12). Diatoms and green algae dominated, each accounting for 45 percent of the phytoplankton community. Density of F. crotonensis was 54,000 cells L^{-1}, which represented an 80-fold increase from January. Fragilaria crotonensis accounted for 1.4 % of the March phytoplankton community.

At the outset of the March experiment concentrations of soluble reactive phosphorus, total soluble phosphorus, nitrate, and reactive silicate in Pigeon Bay were 1.4, 14.8, 387, and 326 μg L^{-1}, respectively. Throughout the course of the experiment concentrations of silica in silica enrichment treatments were maintained at approximately 500 μg L^{-1}. Concentrations of silica in treatments not enriched with silica were depleted from 326 μg L^{-1} on day 0 to less than 50 μg L^{-1} on day 10 and finally to less than or equal to 20 μg L^{-1} on day 20 (Table 10). Concentrations of phosphorus

Figure 13. The response of Pigeon Bay phytoplankton assemblages collected in January, 1984 to increasing light intensity and phosphorus enrichment. P=phosphorus.

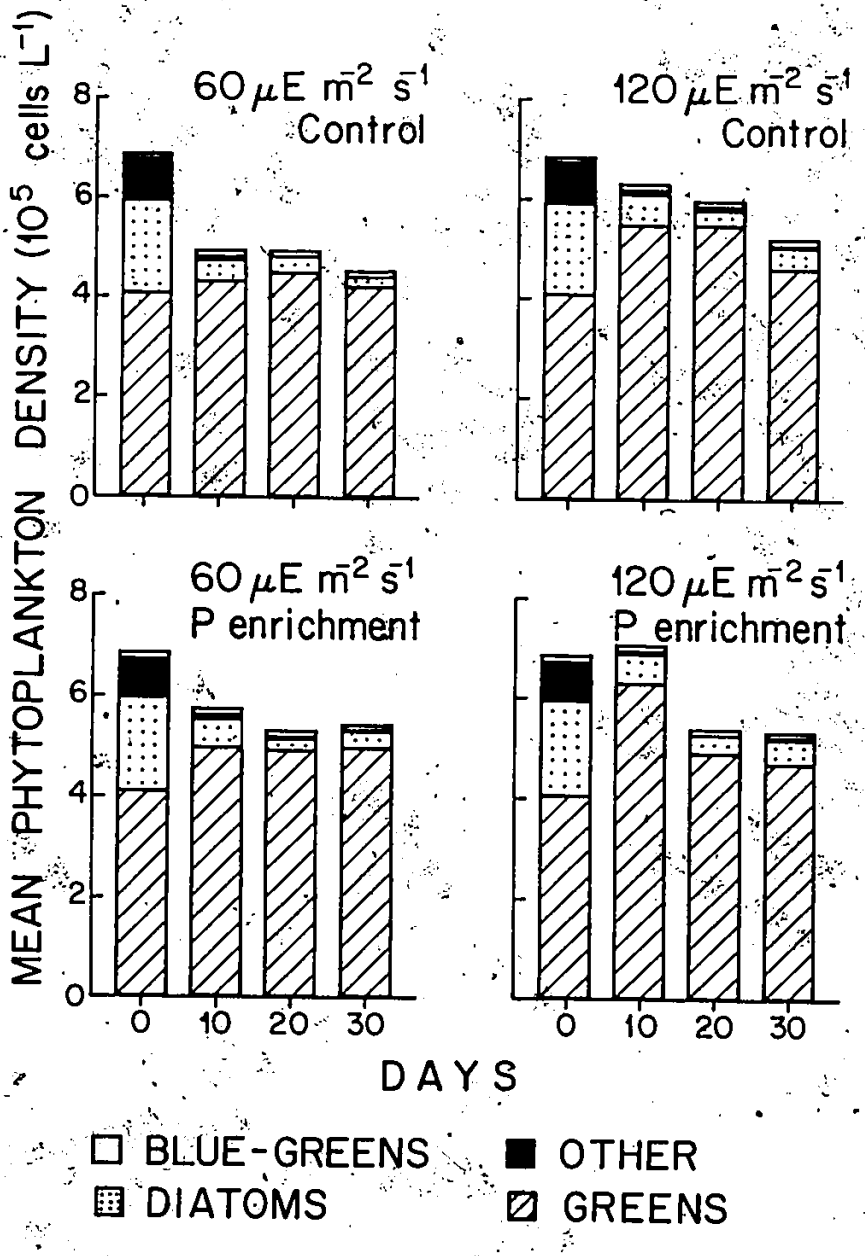

60 μE m⁻² s⁻¹
Control
120 μE m⁻² s⁻¹
Control
60 μE m⁻² s⁻¹
P enrichment
120 μE m⁻² s⁻¹
P enrichment
MEAN PHYTOPLANKTON DENSITY (10⁵ cells L⁻¹)
0
2
4
6
8
0
10
20
30
DAYS
BLUE-GREENS
OTHER
DIATOMS
GREENS

Table 10. Reactive silicate concentrations (μg L^{-1}) in the different treatment bottles throughout the 20-day experiment, March, 1984. ND=no data.

	60 μE m^{-2} s^{-1}				120 μE m^{-2} s^{-1}			
Day	Control	+P	+Si	+P&Si	Control	+P	+Si	+P&Si
0	325.7	325.7	492.4	492.4	325.7	325.7	492.4	492.4
5	ND	ND	641.7	645.6	ND	ND	663.9	639.1
10	49.1	46.5	525.7	515.0	47.8	28.4	526.6	505.1
15	ND	ND	494.3	520.5	ND	ND	496.8	554.7
20	7.8	15.5	558.0	545.9	20.7	13.0	507.1	494.0

in phosphorus enrichment treatments were maintained at approximately 20 $\mu g\ L^{-1}$, while concentrations of phosphorus in treatments not enriched with phosphorus were consistently low ($<5\ \mu g\ L^{-1}$) throughout the experiment (Table 11).

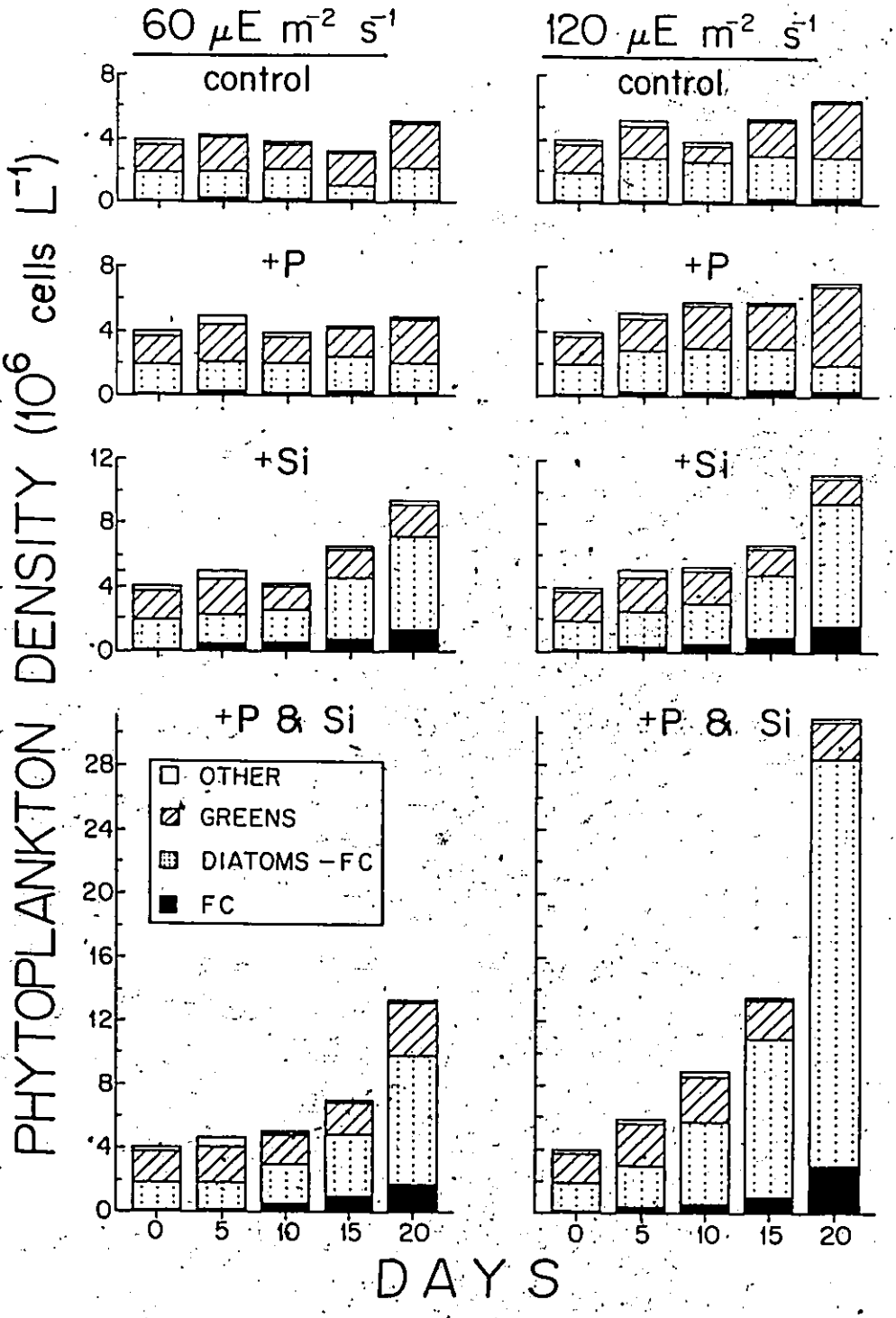
In March, diatoms increased in density over four-fold and percent composition from 45 to 77 % as a result of silica enrichment (Figure 14). No major increase in phytoplankton density was observed in the phosphorus enrichment treatments over the course of the 20-day experiment. All treatments incubated under 120 $\mu E\ m^{-2}s^{-1}$ resulted in higher phytoplankton densities on day 20 than did the same treatments incubated under 60 $\mu E\ m^{-2}s^{-1}$. These data also provide evidence of synergistic effects with 1) phosphorus and silica enrichment; and 2) phosphorus and silica enrichment and increasing light intensity (Figure 14). For example, at an irradiance of 60 $\mu E\ m^{-2}s^{-1}$ the increase in diatom density in the phosphorus and silica enrichment treatment (470 % increase) was greater than the sum of the increases in diatom density of the single nutrient enrichment treatments (+P treatment: 20 % increase; +Si treatment: 330 % increase; sum=350 % increase).

Data from the March experiment also show that not only did density of F. crotonensis increase over 25-fold in 20 days because of silica enrichment, but its percent composition increased approximately 10-fold (Figure 14). This was achieved under both 60 and 120 $\mu E\ m^{-2}s^{-1}$. As with

Table 11. Soluble reactive phosphorus concentrations ($\mu g\ L^{-1}$) in the different treatment bottles throughout the 20-day experiment, March, 1984. ND=no data.

	60 $\mu E\ m^{-2}\ s^{-1}$				120 $\mu E\ m^{-2}\ s^{-1}$			
Day	Control	+P	+Si	+P&Si	Control	+P	+Si	+P&Si
0	1.4	21.4	1.4	21.4	1.4	21.4	1.4	21.4
5	ND	19.7	ND	25.9	ND	19.0	ND	22.8
10	3.4	20.1	3.6	21.4	1.3	20.2	1.5	21.5
15	ND	20.5	ND	20.6	ND	20.1	ND	20.9
20	2.5	21.1	2.0	19.8	1.2	20.5	4.8	20.6

Figure 14. The response of Pigeon Bay phytoplankton assemblages collected in March, 1984 to increasing light intensity and phosphorus and silica enrichment. FC = *F. crotonensis*; Diatoms-FC = Diatoms minus *F. crotonensis*.


60 μE m⁻² s⁻¹
120 μE m⁻² s⁻¹
control
+P
+Si
+P & Si
OTHER
GREENS
DIATOMS – FC
FC
PHYTOPLANKTON DENSITY (10⁶ cells L⁻¹)
DAYS
0
5
10
15
20

the total diatom community; F. crotonensis exhibited a synergistic growth response in the +P&Si treatment incubated at 120 $\mu E\ m^{-2}s^{-1}$ (Figure 14).

4.4 Discussion

4.4.1 Union Water Intake (UWI) Monitoring

Sudden and extreme changes in turbidity in the western basin of Lake Erie affect depth of the photic zone which, in turn, can affect growth of phytoplankton (Chandler 1944). Variation in turbidity, resulting from wind-induced turbulence, is probably the most conspicuous feature of the waters of the western basin; fluctuating from week to week more often than it remains constant (Chandler 1942). High turbidity in the western basin is positively correlated with high suspended solids concentrations (Paul et al. 1982).

Both the UWI Monitoring data and my field sampling data (Section 3) have shown that Fragilaria "pulses" developed in Pigeon Bay when preceded by conditions of low turbidity. It is interesting to note that all nine Fragilaria "pulses" observed between 1977 and 1983 occurred during July, when turbidity was low and water transparency relatively high. It is during the month of July in the western basin that water transparency is normally at seasonal maximum (Appendix 2; Wright 1955; Chandler 1942). Thus, prior conditions of low turbidity appear to be an important factor contributing to the development of large Fragilaria "pulses" in Pigeon Bay. In addition, low light levels will probably limit or co-limit (with a nutrient) growth of Fragilaria every time

turnover results in high turbidity in Pigeon Bay, the frequency determined by meteorological phenomena. Based on several years of study of phytoplankton periodicity in western Lake Erie, Chandler (1944) concluded that low turbidities generally result in high densities of algae and high turbidities generally result in low densities of algae. However, it would be virtually impossible to predict when low or high turbidity levels would occur in Pigeon Bay because it is shallow and therefore particularly subject to unpredictble meteorological conditions.

4.4.2 1983 Nutrient Enrichment Experiments

Both nutrient enrichment experiment data and reactive silicate monitoring data indicate that silica is seasonally an important growth limiting nutrient of F. crotonensis in Pigeon Bay. The conclusion that silica was limiting growth of F. crotonensis in April, September, and October and, by inference, was potentially limiting in June suggests that silica may frequently be an important growth limiting nutrient of F. crotonensis in Pigeon Bay. This is further supported by the fact that during 1983 silica depletion occurred during spring, early summer, and fall in Pigeon Bay.

Nutrient enrichment experiment data also suggest that phosphorus may have been limiting F. crotonensis growth during July. Phosphorus has been shown to be an important growth limiting nutrient of F. crotonensis in Lake Michigan (Stoermer et al. 1978). Wall and Briand (1980) and Tilman

and Sterner (1984) have demonstrated that F. crotonensis is a superior competitor for phosphorus.

4.4.3 1984 Resource Competition Experiments

The January resource competion experiment was performed at 3°C, which, if not limiting growth of most phytoplankton, would be suboptimal (Eppley 1972). No enhancement of growth of F. crotonensis (or total phytoplankton assemblages) resulted from increased light intensity, phosphorus enrichment, or increased light intensity plus phosphorus enrichment during the January experiment. In the western basin of Lake Erie the vernal increase in phytoplankton normally occurs as light conditions improve under the ice during February and March (Chandler 1944). The probable reason why the treatments with increased light intensity did not result in increased phytoplankton densities is that the number of hours of daylight in the light:dark cycle may have been insufficient to stimulate growth. The January experiment was performed under the light:dark cycle (10 hr:14 hr) found at that time. By March, which is the time when Chandler (1944) observed the vernal increase in phytoplankton under the ice, the number of hours of daylight had increased to 12. During 1983, mean incident solar radiation increased from 254 cal $cm^{-2}d^{-1}$ in January to 421 cal $cm^{-2}d^{-1}$ in March. Thus, the trigger for the vernal increase in phytoplankton may be the combination of increasing light intensities and photoperiod.

Little is presently known about the light requirements

of F. crotonensis. In contrast to the January experiment which showed that at 3°C, irradiances of 120 μE $m^{-2}s^{-1}$ and a 10 hr:14 hr photoperiod resulted in no growth of F. crotonensis, earlier laboratory experiments with axenic F. crotonensis (Section 3) showed that at 5°C, irradiances of 120 μE $m^{-2}s^{-1}$ and a 14 hr:10 hr photoperiod resulted in 31 % of the maximum growth rate of this alga at 23°C. Based on these observations and the fact that silica and nitrate were present in concentrations that are normally considered not limiting (Wetzel 1983), it is inferred that F. crotonensis (and total phytoplankton) growth was probably limited by irradiance and temperature throughout the January experiment. Wetzel (1983) has shown that in temperate freshwaters, phytoplankton growth is generally reduced during winter when both light and temperature are low.

In March, when the phytoplankton community was 45 % diatoms and initial reactive silicate concentration was 325 μg L^{-1}, natural phytoplankton assemblage growth was limited by supplies of silica. Springtime silica limitation appears to be a common occurrence in Pigeon Bay (Section 2).

All treatments incubated under 120 μE $m^{-2}s^{-1}$ resulted in higher phytoplankton densities on day 20 than did the same treatments incubated under 60 μE $m^{-2}s^{-1}$. If an irradiance of 60 μE $m^{-2}s^{-1}$ is representative of in situ irradiance during turnover in Pigeon Bay, then growth of phytoplankton was light limited. Further evidence for light limitation of phytoplankton growth in Pigeon Bay is the very

high turbidity levels reported from there by the Union Water Intake (7-day average: 23.07 FTU; one of the highest weekly averages reported during the preceding year). Lean and Nalewajko (1979) have also concluded that phytoplankton growth was light limited at a non-stratified station in western Lake Erie due to high turbidity resulting in a 1 % light level of 2.2 m.

These data also provide evidence of synergistic effects with: 1) phosphorus and silica enrichment; and 2) phosphorus and silica enrichment and increased irradiance. Synergistic effects in +P & Si treatments may have been the result of changes in supples of nutrients or nutrient ratios which are believed to play important roles in structuring phytoplankton communities (Tilman et al. 1982). The synergistic effects of phosphorus and silica enrichment and increasing irradiation show that not only can Pigeon Bay phytoplankton be both light and nutrient limited, but that relieving this dual limitation can result in substantial growth of phytoplankton (even at 5°C). Synergistic effects of light and nutrient on growth of phytoplankton have also been documented in the laboratory by Rhee and Gotham (1981).

Data from the March experiment also show that not only did density of F. crotonensis increase over 25-fold in 20 days due to silica enrichment, but percent composition increased approximately 10-fold. This indicates that F. crotonensis was silica limited. The increase in percent composition of F. crotonensis as a result of silica

enrichment provides strong evidence that this species can sucessfully compete with other diatoms for silica. Wall and Briand (1980) observed similar responses of F. crotonensis to nutrient enrichment and concluded that it was a superior exploitative competitor for nutrients. Several recent studies indicate that F. crotonensis is a superior competitor for silica (Tilman et al. 1982; Lovstad 1984; Tilman and Sterner 1984). Stoermer et al. (1978) corroborate the fact that F. crotonensis is a superior exploitative competitor for major nutrients with data from nutrient enrichment experiments performed on natural phytoplankton assemblages from Grand Traverse Bay, Lake Michigan. They found that F. crotonensis became the dominant taxon in all 15 treatments (incorporating various enrichments of phosphorus and nitrogen), composing from 35.6 % of total cell numbers (51.2 % of total diatoms) to 65.2 % (74.6 % of total diatoms).

The synergistic growth response of F. crotonensis in the +P & Si treatment incubated at 120 $\mu E\ m^{-2}s^{-1}$ shows that F. crotonensis can also be both light and silica limited and that relieving this dual limitation can result in substantial growth. Further, at lower light (60 $\mu E\ m^{-2}s^{-1}$) F. crotonensis was able to increase its density 25-fold and its percent composition 13-fold in 20 days as a result of silica enrichment. This further demonstrates that F. crotonensis is adapted to a low light environment (Section 3). In Pigeon Bay, F. crotonensis is probably exposed to a

low light environment a high percentage of the time due to the turbid (Section 4) and productive (Section 2) nature of these waters. Chandler (1942) has shown that turbidity in the western basin significantly reduces light available to phytoplankton. He found that low turbidity enabled a "bloom" of phytoplankton to develop under the ice and high turbidity prevented spring "blooms".

5. Sinking and Resuspension of *F. crotonensis*

5.1 Introduction

The seasonal variation of phytoplankton as a whole and of its particular component species is controlled by one or more physical, chemical, or biological factors (Hutchinson 1967). In certain systems, the combination of sinking and resuspension of phytoplankton is believed to be the most important factor in phytoplankton survival and success (Margalef 1978). The purpose of this study was to quantitatively determine sinking rates of *F. crotonensis* in the laboratory and document the vertical distribution of *F. crotonensis* in Pigeon Bay under varying meteorological conditions. Such information should provide insight into the role of sinking and resuspension in the biology of *F. crotonensis* which should lead to a better understanding of the large *F. crotonensis* "pulses" observed in Pigeon Bay in recent years (Nicholls et al. 1980).

5.2 Materials and Methods

5.2.1 Laboratory Sinking Rates

Unialgal cultures of *F. crotonensis* were isolated from Lake Erie and maintained in exponential growth phase in freshwater "WC" media (Guillard and Lorenzen 1972) at 16°C and 100 $\mu E\ m^{-2}s^{-1}$ illumination on a 14 hr:10 hr light:dark cycle. To estimate sinking rates of *F. crotonensis*, filtered (0.45 μm Millipore filter) western Lake Erie water (16°C) was placed in a 50 ml settling chamber (height: 110 mm) mounted on an inverted microscope and inoculated with

exponential growth phase F. crotonensis. The precise inoculation procedure was to slowly expel 3-5 drops of F. crotonensis culture from a Pasteur pipette onto the surface of the filtered lake water. Four to six quadrats, chosen at random, were examined microscopically every 10 minutes for 8 hours to enumerate the chains. Both chain area and number of cells per chain were recorded. Sinking rates were calculated as m day^{-1}.

5.2.2 Vertical Distribution of F. crotonensis in Pigeon Bay

On 20 June 1983, following five days of low wind velocities, discrete phytoplankton samples were collected with a Van Dorn bottle from 0.5, 2.0, and 3.5 m at the Pigeon Bay station (see Section 2). Phytoplankton samples were immediately preserved in Lugol's Solution. Oxygen (mg L^{-1}) and temperature (°C) profiles were determined with a Y.S.I. Model 57 oxygen meter. Water transparency was measured with a Secchi disk. On 30 June 1983, two days after a storm passed over Pigeon Bay, precisely the same sampling program was performed to establish the vertical distribution of F. crotonensis, temperature, and oxygen. Secchi disk was again measured. In the laboratory, cells of F. crotonensis were enumerated using an inverted microscope (Utermöhl 1958). A minimum of 150 chains of F. crotonensis were enumerated from at least two subsamples from each preserved sample.

5.3 Results

5.3.1 Laboratory Sinking Rates

Fragilaria crotonensis is a large (length: 40-150 μm), chain-forming (length: up to 3 mm) diatom. One consequence of chain formation in diatoms is that it usually increases the sinking rate (Smayda and Boleyn 1965, 1966a, 1966b). Laboratory experiment data from this study confirmed that long chains of F. crotonensis sink faster than short ones (Table 12). The most obvious reason for this is that the total area of the colony is smaller than the sum of the areas of the isolated cells (Sournia 1981). The mean laboratory sinking rate of all F. crotonensis in this study was 6.32 m day^{-1}.

5.3.2 Vertical Distribution of F. crotonensis in Pigeon Bay

On 20 June 1983, following five days of low wind velocities, the Secchi disk measurement in Pigeon Bay was greater than 2 m and there was slight stratification based on oxygen and temperature profiles (Figure 15). Density of F. crotonensis increased with increasing depth from approximately 6,000 cells L^{-1} at 0.5 m to over 30,000 cells L^{-1} at 3.5 m. High winds from a storm over Pigeon Bay on 27-28 June 1983 caused complete mixing of the basin as evidenced by uniform temperature and oxygen concentration with depth and a low Secchi disk value on 30 June 1983. Resuspension was evident at this station following the storm since there was a 600 % increase in the density of F. crotonensis at 0.5 m (Figure 15).

5.4 Discussion

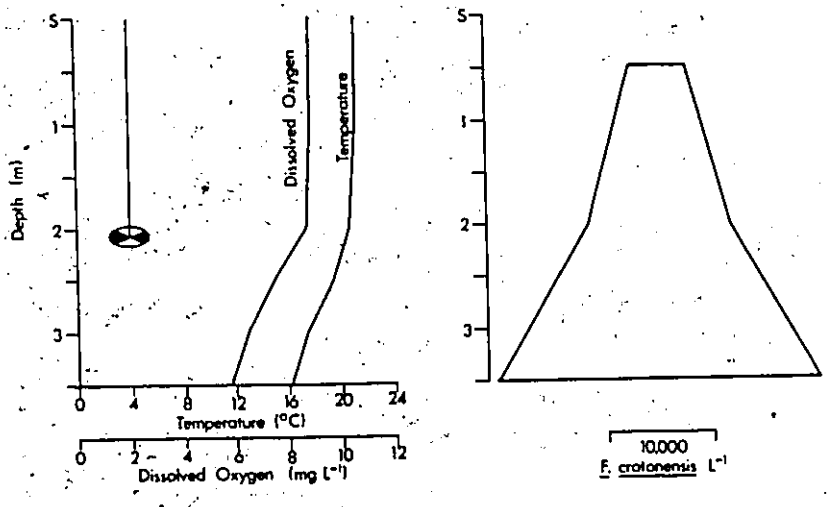
Silica is a relatively heavy substance accumulated in

Table 12. Influence of chain size on labortory sinking rates (m day^{-1}) of exponential growth phase F. crotonensis.

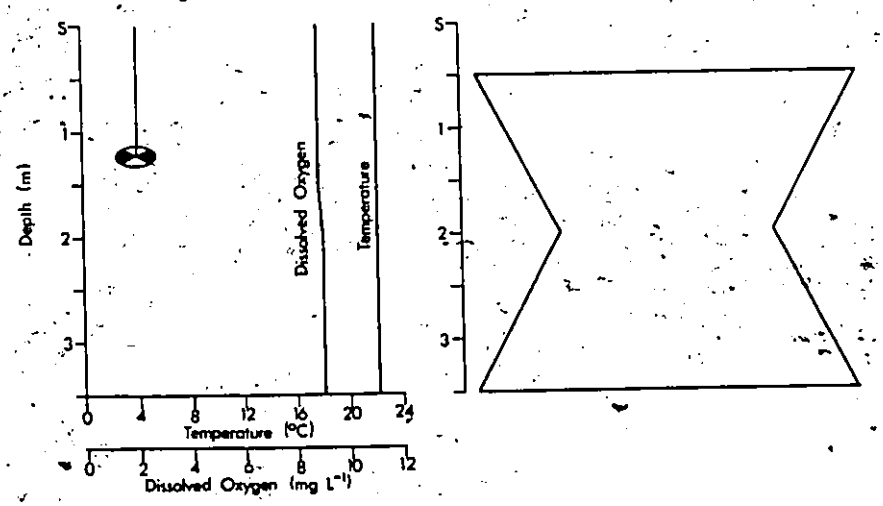
Area of Chain (mm^2)	Range of Cells/Chain	n	Sinking Rate ($m\ day^{-1}$)	Standard Deviation
0-0.0010	1-4	12	1.10	0.55
0.0011-0.0020	4-12	22	4.18	4.23
0.0021-0.0050	4-23	29	3.95	4.19
0.0051-0.0100	10-25	34	7.15	5.87
0.0101-0.0200	12-47	33	6.51	5.86
0.0201-0.0300	12-60	10	9.82	6.64
0.0301-0.0400	75-103	4	15.84	0.00
0.0401-0.0500	91-105	6	11.88	4.34
> 0.0500	114-380	6	12.45	5.44
X			6.32	5.82

Figure 15. The vertical distribution of dissolved oxygen (mg L^{-1}), temperature (°C), and *E. crotonensis* (cells L^{-1}) in Pigeon Bay before and after a storm event.

20 June 1983



30 June 1983



large quantity in diatoms enhancing sinking in undisturbed water (Walsby and Reynolds 1980). Sinking rates in diatoms have been shown to be influenced by culture age, cell size, colony size and mode of formation (Smayda and Boleyn 1965, 1966a), and physiological state (Titman and Kilham 1976).

Experiments in this study have shown that the mean laboratory sinking rate of exponential growth phase F. crotonensis is approximately 6 m day^{-1} (Table 12). This could lead to a high in situ sinking rate if the shallow (mean depth: 7.6 m) and turbid (Paul et al. 1982) waters of the western basin are undisturbed by winds. From laboratory experiments, Fritz (1935) reported sinking rates of 5.1 m day^{-1} at 6°C and 9.1 m day^{-1} at 29°C for F. crotonensis. Based on actual movement of diatoms in Lake Constance, Grim (1939) observed F. crotonensis sinking at rates up to 7 to 9 m day^{-1}. These maximal sinking rates were achieved only in the relatively non-turbulent waters of the hypolimnion; in the epilimnion, because of turbulence, sinking rates were markedly less. In Crose Mere, Reynolds (1976) used a simple trapping method to estimate the sinking rate of F. crotonensis at 0.21-0.65 m day^{-1}. Earlier, Reynolds (1973) measured in vitro sinking rates of F. crotonensis of different sizes and conditions at 0.2-0.3 m day^{-1}. I believe these lower estimates of sinking rate may reflect the differences in methods employed. In the trapping method, traps were set in 1 m intervals and retrieved twice per week to investigate in situ sinking

rates. This sampling frequency may have been too low to accurately measure sinking rates and was potentially affected by resuspension of cells. Reynolds measured *in vitro* sinking rates of individual chains of *F. crotonensis* in a flat sided cuvette through a laterally mounted inverted microscope. A pair of parallel lines were constructed in the eyepiece to time the passage of a chain across a measured distance. The potential for error in using such a technique seems great because of the short distance used to measure sinking rates. Problems such as drag near chamber walls and thermal currents from a microscope lamp would only be magnified in using such a short distance to estimate sinking rates. However, even if *F. crotonensis* sank at approximately 1 m day^{-1} in Pigeon Bay during undisturbed conditions, this could be significant because of the high turbidity (Paul et al. 1982) and frequently shallow photic zone (range of Secchi disk values in 1983: 0.8-2.2 m).

Sinking produces potential disadvantages for phytoplankton in leading to their removal from the photic zone (Walsby and Reynolds 1980). On the other hand, it may produce potential advantages in accelerating rates of nutrient uptake (Smayda 1970). The disadvantages of sinking out of the photic zone can be overcome through resuspension via physical movements of water. Such movements include turbulence, wind induced patterns, thermic convection, and various water transports. According to Smayda (1970), velocities of wind-driven convection cells exceed the

maximum sinking rates of most phytoplankton by 2 or 3 orders of magnitude. For chain-forming diatoms such as F. crotonensis, large surface areas will enhance resuspension via physical movements of water. Because of this physical aid, F. crotonensis may move up and down the water column and may benefit temporarily from deeper more nutritive strata. Sinking and resuspension of F. crotonensis are illustrated by data on its vertical distribution before and after a storm event. Following the storm which passed over Pigeon Bay, densities of F. crotonensis increased approximately 600 % in surface waters (0-5 m). This shows that "seeding" of the photic zone with F. crotonensis can occur in Pigeon Bay. Given the ability of many diatoms to survive in the dark at low temperatures, Malone (1980) believes that the development of diatom blooms could reflect the availablity of "seed" populations in the benthos that are resuspended during mixing events. However, it must be remembered that high winds have the ability to increase turbidity substantially in Pigeon Bay which may result in light limitation of F. crctonensis growth (Section 4).

Highest field density of F. crotonensis in the surface mixed layer during the 1983 field season was 950,000 cells L^{-1} on July 26 (Section 3). This peak density occurred when there was slight thermal stratification (Appendix 2), low turbidity (7-day average: 2.86 FTU), relatively high light penetration (Secchi disk: 2.2 m; highest observed in 1983), and high nutrient concentrations (Si: 871 µg L^{-1}; soluble

reactive phosphorus: 5.5 µg L^{-1}). During this time the depth of the surface mixed layer was about 2 m (Appendix 2). This suggests that the development of large F. crotonensis populations during the summer months in Pigeon Bay appears to be dependent upon three major factors:

1) the presence of slight thermal stratification with concomitant wind velocities sufficient to keep F. crotonensis circulating in the surface mixed layer (7-day average wind speed preceeding 26 July 1983 density of 950,000 cells L^{-1}: 10.4 km hr^{-1});
2) the presence of low turbidities (< 5 formazin turbidity units) with concomitant irradiance sufficient for F. crotonensis growth; and,
3) adequate supplies of nutrients (primarily Si and P).

Once such physicochemical conditions exist in Pigeon Bay it appears that F. crotonensis can grow rapidly because it is adapted to a low light environment (Section 3) and is a superior exploitative competitor for nutrients (Section 4).

Maximum phytoplankton growth rates are known to vary inversely with cell size (Banse 1976) and this, together with evidence that smaller cells are superior in nutrient uptake to large cells (Smith and Kalff 1982), has been assumed to show that smaller phytoplankton are superior to larger ones in nutrient competition (Schlesinger et al. 1981). If this is true, then how can large, chain-forming F. crotonensis reach such high densities in Pigeon Bay? One possible explanation is that in the turbulent waters of

Pigeon Bay *F. crotonensis* (with its high sinking rate) may have a competitive advantage over small phytoplankton (with low sinking rates) because sinking may bring cells into regions of higher nutrient concentrations, thereby increasing the potential for nutrient uptake (Smayda 1970; Titman and Kilham 1976). An ability to store phosphorus may also provide a mechanism by which *F. crotonensis* grow faster than small phytoplankton in nature (Malone 1980). Further, variable nutrient environments or environments which permit nutrient uptake but little growth (e.g. nutrient rich, low light environments such as Pigeon Bay) could set the stage for bursts of exponential growth during which large cells grow on nutrient reserves at higher rates than small cells for one or two generations (Malone 1980). Lean (1984) has demonstrated that large phytoplankton can take advantage of sudden increases in phosphorus and store enough in a few minutes to meet their metabolic needs for several generations.

Another morphological characteristic of chain-forming *F. crotonensis* is twisting or turning of the chains which results in a rotary-like motion during sinking and resuspension. Smayda (1970) believes the advantages of rotary-like motion to phytoplankton are that it would tend to break up micro-gradients of nutrients.

If phytoplankton such as *F. crotonensis* were to remain stationary in the water column, rapid nutrient uptake would lead to nutrient limitation (Smayda 1970). This suggests

that movement within the water column is desirable to promote nutrient acquisition. As Smayda (1970) so succinctly stated:

> "The problem for phytoplankton is not to float, but to sink or rise and rotate."

In the Pigeon Bay waters of western Lake Erie, F. crotonensis's high sinking rate and large surface area, together with its apparent adaptation to a low and variable light environment (Section 3) and its superior exploitative ability to compete for nutrients (Section 4), may lead to a competitive advantage over other phytoplankton during periods of turbulence.

6. Predation and Parasitism on *F. crotonensis*

6.1 Introduction

Density and relative abundance of *F. crotonensis* in Pigeon Bay is believed to be closely correlated to the occurrence of physical processes (Section 5). Resource competition theory predicts that the relative abundance of different species within the phytoplankton community is the result of varying abilities to acquire and utilize nutrients (Titman 1976; Tilman et al. 1982). Another theory suggests that nutrient competition should be considered as having little effect on species competition and succession, which is then thought to be controlled by other processes, such as selective grazing (Maestrini and Bonin 1981). The purpose of this study is to investigate the potential effects of predation and parasitism on *F. crotonensis*. Such information should lead to a better understanding of the anomalous *F. crotonensis* periodicity in Pigeon Bay in recent years (Nicholls et al. 1980).

6.2 Materials and Methods

On 31 May 1983 live crustacean zooplankton were collected from the Pigeon Bay station for microscopic observation of feeding behavior. Zooplankton were collected by vertical hauls using a silk no. 10 mesh (158 µm) Wisconsin Net. The zooplankters were immediately brought to shore, placed in watch glasses, and feeding behavior observed for five minutes under a stereozoom microscope to determine if they had been damaged through collection or

transfer. If the animals appeared to be swimming and feeding normally on phytoplankton present, laboratory reared F. crotonensis were inoculated into the watch glass. The feeding behavior of the zooplankton was then observed for another 15 minutes for evidence of grazing on chains of F. crotonensis. Observations were made on a minimum of three specimens of all common crustacean zooplankton.

No field observations of parasitism or experiments on planktivorous gizzard shad (Dorosoma cepedianum) predation were performed. Pertinent literature is reviewed.

6.3 Results and Discussion

Zooplankton grazing on phytoplankton has often been invoked as a significant factor in the decline of algal populations and as contributing to seasonal succession (Hutchinson 1967). Some studies have suggested that at certain times of the year, zooplankton can totally graze the trophogenic zone in a day (Hargrave and Geen 1970; Enright 1969).

Zooplankton exhibit a broad range of feeding habits (McNaught et al. 1980). Cladocerans filter feed on a broad size range of particles, the upper limit to the size of the particle ingested whole being directly related to the size of the cladoceran (Burns 1968). Copepods are believed to be actively selective and highly specialized. Apparently some copepods can switch feeding modes, feeding raptorially when large particles are abundant and filtering when there are more small particles (McQueen 1970). Particle modification

by copepods, largely due to breakage of cells and fragmentation of colonial algae and chain-forming diatoms, has been observed by O'Connors et al. (1976) and Richman et al. (1980). Planktonic rotifers feed largely on seston particles. Most food particles ingested by rotifers are small (less than 12 μm in diameter), although larger cells (up to approximately 50 μm) are sometimes taken (Wetzel 1983).

The zooplankton community of western Lake Erie is diverse, with 14 species of Cladocera (Watson and Carpenter 1974), 19 species of Copepoda (10 calanoid, 7 cyclopoid, and 2 harpacticoid; Robertson and Gannon 1981), and 25 species of Rotifera (Watson 1974) reported. On 31 May 1983 live crustacean zooplankton were collected from Pigeon Bay for microscopic observation of feeding behavior. Observations of at least three specimens of Bosmina longirostris, Bubosmina coregoni, Ceriodaphnia sp., Diacyclops thomasi (adults and copepodids), and Diaptomus spp. (adults and copepodids) provided no evidence of grazing on chains of F. crotonensis. Adult and immature copepods would swim up to a chain of F. crotonensis, pause, and then swim away. Raptorial feeding copepods are known to feed on F. crotonensis based on observations of fragments of F. crotonensis in their guts (Kreis et al. 1983). However, the quantitative extent of such grazing on F. crotonensis is not known. Chains of F. crotonensis were too large for the cladoceran filter feeders. The feeding of rotifers was not

observed because chains of F. crotonensis far exceed the upper size limit (50 μm) of food particles capable of being consumed by rotifers (Wetzel 1983). One other important observation is that highest F. crotonensis biomass in Pigeon Bay typically occurs in July (Section 4; Nicholls et al. 1980) when zooplankton biomass is highest in western Lake Erie (Watson and Carpenter 1974). The occurrence of highest F. crotonensis biomass during periods of highest zooplankton biomass suggests that zooplankton grazing on F. crotonensis in western Lake Erie is probably insignificant. Reynolds (1973) concluded that grazing on larger diatoms such as F. crotonensis is probably insignificant in Crose Mere, England.

In phytoplankton, large size may be an adaptation against grazing (Hutchinson 1967). If no significant grazing on F. crotonensis occurs in Pigeon Bay, this might lead to a competitive advantage for F. crotonensis over other phytoplankton, depending upon the prevailing physicochemical conditions. Malone (1980) has reported that increases in net-phytoplankton biomass (usually diatoms) in coastal environments occur most frequently and are greatest in amplitude when preceded by a period of minimum grazing pressure.

Predation by filter-feeding fish may also influence F. crotonensis periodicity in Pigeon Bay. Gizzard shad (Dorosoma cepedianum) is an important component of the fish community in western Lake Erie and is most abundant in the

shallow waters around the periphery of the basin, especially in protected bays and mouths of tributaries (Bodola 1966). Food of gizzard shad varies widely with season and locality but consists mostly of phytoplankton and zooplankton. Bodola (1966) noted that during June, July, and August diatoms were one of the more plentiful items in stomach contents of gizzard shad from the western basin. Fragilaria has been reported from 63 % of gizzard shad foregut samples from two Ozark, Arkansas reservoirs (Baker and Schmitz 1971). During summer months, Fragilaria was one of the three dominant algae ingested by gizard shad. Drenner et al. (1984) have shown that ingestion rates of filter-feeding gizzard shad increase as a function of particle size, leveling off at 60 µm. Thus, because of the abundance of gizzard shad in the western basin and the fact that F. crotonensis (by virtue of its large size) can potentially be ingested at maximum rate, gizzard shad predation may supress development of a F. crotonensis "pulse" or contribute to termination of a F. crotonensis "pulse". Further research on the impact of gizzard shad predation on development and termination of F. crotonensis "pulses" is warranted.

Fungal parasitism is another type of species interaction which may in turn lead to seasonal succession. Although many freshwater algae are known to be susceptible to fungal parasites, the control which the parasites effect upon the growth of hosts remains largely unknown (Wetzel

1983). Rhizophydium fragilariae, a chytrid fungus, is known to parasitize F. crotonensis (Canter and Jaworski 1982). Parasitism is believed to increase in eutrophic waters (Wetzel 1983) and may delay the time of algal maximum, decrease the size of algal maximum, or even cause a decline in numbers if the parasite reaches epidemic proportions (Reynolds 1973).

Parasitism which reduces a population of a dominant phytoplankter or its potential is but one way to influence seasonal succession. Another way to influence seasonal succession was identified by Van-Donk and Ringelberg (1983) who showed that fungal parasitism can diminish a population of Asterionella formosa which in turn favors development of F. crotonensis in Lake Maarsseveen, Netherlands.

No conclusions can be drawn from this study on the role of fungal parasitism in the waxing and waning of F. crotonensis in the Pigeon Bay waters of western Lake Erie. Suffice it to say, little is known of its quantitative significance.

7. Summary and Conclusions

The primary purpose of this study was to identify the factors which have contributed to the recent increased biomass (primarily summer "pulses") of F. crotonensis in the Pigeon Bay waters of western Lake Erie. Photosynthetically active radiation, bioavailable nutrients, water turbulence, and temperature have major influence on the seasonal succession of F. crotonensis (Figure 16). The factors acting on photosynthetically active radiation, bioavailable nutrients, water turbulence, and temperature are complex and highly variable reflecting the productive and dynamic nature of Pigeon Bay.

Availability of light appears to be a factor potentially limiting growth of F. crotonensis during all seasons (Table 13). During winter when ice is present on the bay, F. crotonensis can rapidly sink out of the photic zone and growth appears to be limited by both light and temperature. The early spring phytoplankton "pulse" (primarily diatoms other than F. crotonensis) in Pigeon Bay begins under the ice when temperatures are still low and nutrient concentrations (phosphorus and silica) are relatively high. When the ice moves off Pigeon Bay turnover occurs and F. crotonensis is resuspended. Early on in spring turnover F. crotonensis appears to grow well because it is eurythermal, adapted to a low and variable light environment, and is a superior exploitative competitor for nutrients (silica and phosphorus). This is supported by

Figure 16. A schema of the relationship between major physicochemical factors affecting growth of F. crotonensis in the Pigeon Bay waters of western Lake Erie. For the sake of simplicity certain interrelationships of secondary importance have been omitted.

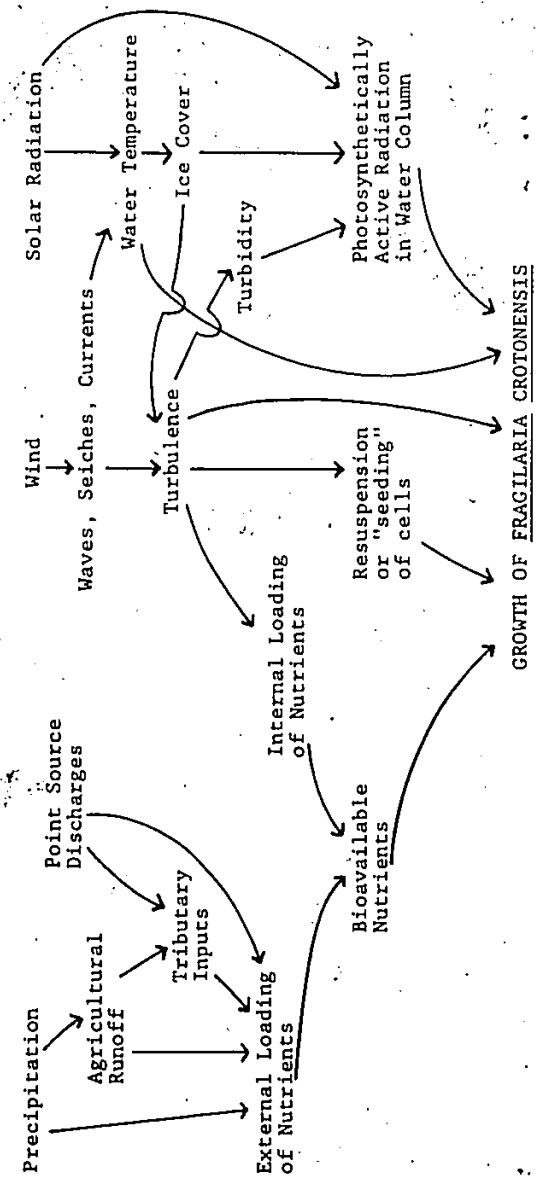

Solar Radiation
Water Temperature
Ice Cover
Photosynthetically Active Radiation in Water Column
Turbidity
Wind
Waves, Seiches, Currents
Turbulence
Resuspension or "seeding" of cells
GROWTH OF FRAGILARIA CROTONENSIS
Internal Loading of Nutrients
Point Source Discharges
Tributary Inpucs
Agricultural Runoff
Precipitation
External Loading of Nutrients
Bioavailable Nutrients

Table 13. A summary of factors potentially limiting growth of F. crotonensis in the Pigeon Bay waters of western Lake Erie. Incident solar radiation data were collected by Environment Canada-Atmospheric Environmental Services at the Harrow Research Station, Ontario. FC=F. crotonensis.

Date	Water Temp. (^{o}C)	$\bar{x}$ Monthly Incident Solar Radiation (cal $cm^{-2}d^{-1}$)	Secchi Disk (m)	Turbidity 7-day ave.(FTU)	Soluble Reactive P ($\mu g\ L^{-1}$)	Reactive Si ($\mu g\ L^{-1}$)	Nitrate ($\mu g\ L^{-1}$)	FC (cells L^{-1})	Nutrient Enrichment Experiment Results	Factors Potentially Limiting FC Growth
27 Apr. 1983	5.5	254.1	1.0	11.8	1.5	13.9	277.0	260,700	enhancement of FC growth with Si	light, Si, temperature
17 May 1983	8.0	421.1	1.5	4.6	4.6	13.4	215.0	44,000	no significant FC growth (Si may be important)	light, Si, temperature
21 June 1983	22.0	555.1	2.1	3.4	< 1.0	75.1	370.1	8,700	no data because of attached algal growth (Si may be important)	light, Si
26 July 1983	23.4	578.7	2.2	2.9	5.5	871.4	171.0	950,000	no significant FC growth (P may be important)	light, P
15 Aug. 1983	24.2	500.5	1.0	10.4	< 1.0	665.1	182.6	126,700	no significant FC growth (P or Si may be important)	light, P or Si
26 Sept. 1983	17.0	403.1	1.0	11.2	< 1.0	161.0	123.1	219,900	enhancement of FC growth with Si	light, Si
26 Oct. 1983	11.3	189.0	0.8	15.8	4.6	227.1	218.8	65,000	enhancement of FC growth with Si	light, Si, temperature
25 Jan. 1984	1.0	90.1	1.5	4.1	6.2	912.4	260.3	700	no significant FC growth	light, temperature
26 Mar. 1984	2.0	159.6	0.8	23.1	1.4	325.7	386.7	54,000	enhancement of FC growth with Si	light, Si, temperature

observations of Reynolds (1973) that in many temperate lakes, F. crotonensis tends to be one of the predominant phytoplankters in spring because it is able to grow faster than its competitors when the water is still cold. As turnover continues depletion of silica in Pigeon Bay results in silica limitation of F. crotonensis growth. Pigeon Bay provides a suboptimal temperature environment for growth of F. crotonensis as long as water temperatures remain below 17°C.

In general, as water temperatures increase with the onset of summer, slight thermal stratification can occur in Pigeon Bay which, if coupled with no or very low turbulence, allows F. crotonensis to sink out of the photic zone. Harris (1980) notes that phytoplankton which lack flagella or gas vacuoles to maintain buoyancy are dependent on wind induced turbulence to grow in the surface mixed layer. Thermal stratification can also lead to phosphorus depletion in surface waters (Lean et al. 1983) and possible phosphorus limitation of F. crotonensis growth. Turbulent interruptions of stratification can occur frequently and at any time (dependent on winds). High wind velocities can substantially increase turbidity which can result in light limitation of F. crotonensis growth. Fragilaria crotonensis must keep suspended in the photic zone (by low turbulence or convection) with concomitant low turbidity levels (< 5 FTU) to set the stage for a F. crotonensis "pulse". The highest field density of F. crotonensis in this study occurred

during slight thermal stratification on 26 July 1983 when the Secchi disk measurement was 2.2 m (highest recorded) and the 7-day average turbidity was 2.86 FTU (lowest recorded) (Table 13). It is during the month of July that incident solar radiation and water transparency are normally at seasonal maxima in western Lake Erie (Wright 1955; Chandler 1942).

Summer F. crotonensis "pulses" are also dependent upon supplies of bioavaiable nutrients. During summer (i.e. normally July), replenishment silica concentrations (via dissolution of biogenic silica and/or external loading) appears to be a necessary requirement for a F. crotonensis "pulse". Internal loading of phosphorus can replenish soluble reactive phosphorus supplies in Pigeon Bay. In addition, internal phosphorus pools in F. crotonensis may be maintained via luxury consumption. Malone (1980) believes that an ability to store phosphorus may provide a mechanism by which large phytoplankton grow faster than small phytoplankton in nature. Further, variable nutrient environments or environments which permit phosphorus uptake but little growth (e.g. phosphorus rich, low light environments such as Pigeon Bay) could set the stage for bursts of exponential growth during which large cells grow on nutrient reserves at higher rates than small cells for one or two generations.

All nine F. crotonensis "pulses" observed in Pigeon Bay between 1977 and 1983 occurred during July when water

temperatures were optimal for growth and incident solar radiation and water transparency were at seasonal maxima. It is hypothesized that the emergence of large F. crotonensis populations during these summer months (i.e. July) in Pigeon Bay is dependent upon three major factors:

1) adequate supplies of nutrients (Si: via dissolution of biogenic silica and/or external loading; P: via luxury consumption and/or internal or external loading);
2) the presence of slight thermal stratification with concomitant wind velocities sufficient to keep F. crotonensis circulating in the surface mixed layer; and,
3) the presence of low turbidities (<5 FTU) with concomitant irradiance sufficient for growth.

Once such physicochemical conditions exist in Pigeon Bay it appears that F. crotonensis can grow rapidly (because it is adapted to a low light environment and is a superior exploitative competitor for nutrients) and achieve densities as high as 950,000 cells L^{-1} (Table 13). Fragilaria crotonensis "pulses" in Pigeon Bay may be terminated by lack of wind (which allows F. crotonensis to sink out of the photic zone), high wind velocities (which increase turbidity and result in light limitation), or by depletion of nutrients (i.e. P or Si). Zooplankton grazing on F. crotonensis is probably insignificant in Pigeon Bay. Fragilaria crotonensis's chain-forming morphology and large

size may be an adaptation for resistance to zooplankton grazing which may contribute to a competitive advantage over other phytoplankton under certain conditions. The role of fungal parasitism and planktivorous fish predation in the waxing and waning of F. crotonensis in Pigeon Bay is not known.

A scenario, such as the one presented above, for the development of F. crotonensis "pulses" during the summer in Pigeon Bay may complement Nicholls et al. (1980) theory that highest annual Fragilaria biomass occurs during years with low water levels. Their annual Fragilaria biomass data are strongly influenced by summer F. crotonensis "pulses". Low water levels in western Lake Erie may enhance the physicochemical conditions necessary for development of F. crotonensis "pulses" (low water levels may enhance F. crotonensis "seeding" of the photic zone, increase the probability of F. crotonensis suspension in the surface mixed layer, and/or maintain adequate supplies of nutrients via internal loading) which strongly influence annual Fragilaria biomass.

In the fall in Pigeon Bay, growth of F. crotonensis appears to be limited by light and silica (Table 13). Once water temperatures fall below 17°C growth of F. crotonensis is probably again temperature limited.

In discussing the factors which contribute to F. crotonensis "pulses" in Pigeon Bay one must also maintain a historical perspective. For example, substantial chemical

and biological changes have occurred in Pigeon Bay since the late 1960's (Nicholls et al. 1980). Summer nitrate:soluble reactive phosphorus ratios in Pigeon Bay have increased from approximately 4 in the late 1960's to over 40 in the late 1970's and early 1980's. During this same period the summer phytoplankton community shifted from being primarily nitrogen limited to being primarily phosphorus limited. Nicholls et al. (1980) have shown that blue-green algal biomass has declined during this period while diatom biomass has apparently increased. It has been suggested that blue-green algae are generally better nitrogen competitors, but poorer phosphorus competitors, than other groups of algae indicating that blue-green algae should typically be dominant in lakes with low N:P ratios and rare in lakes with high N:P ratios (Tilman et al. 1982; Smith 1983). In general, diatoms are thought to be efficient phosphorus competitors indicating that they should be dominant in lakes with high N:P ratios (Tilman et al. 1982). Based on Nicholls et al. (1980) historical trend data from Pigeon Bay, F. crotonensis appears to be a more important component of the summer phytoplankton community in the late 1970's. It is possible that this increase in summer nitrate:soluble reactive phosphorus ratios in Pigeon Bay has provided a more optimal environment for growth of F. crotonensis. In the laboratory, Rhee and Gotham (1980) have shown that F. crotonensis has a relatively high optimum N:P ratio (25) which is consistant with increased F. crotonensis abundance

with increased N:P ratios in Pigeon Bay.

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9. Appendices

9.1 Appendix 1: Isolation and Purification of F. crotonensis

On 10 Nov. 1982 a nearshore water sample was collected from 3 km off the north shore of Lake Erie at Wheatley, Ontario (N420223.2; S822622.1). Using a sterile pipette technique (Guillard 1973) a single clone of Fragilaria crotonensis was isolated in freshwater "WC" media (Guillard and Lorenzen 1972) on 21 Nov. 1982.

Following establishment of an unialgal culture, at least twenty chains of live F. crotonensis were removed from the culture and mounted on a glass microscope slide. The F. crotonensis were then viewed at 1000X for the presence of fungal parasites. If no parasitic fungi were observed on any F. crotonensis, purification procedures were initiated.

Hoshaw and Rosowski (1973) have identified the following four methods of purification yielding axenic cultures: centrifugation, ultrasonic treatment, antibiotic treatment, and potassium tellurite treatment. To purify F. crotonensis into axenic culture, both ultrasonic treatment and antibiotic treatment were attempted.

Ultrasonic treatment uses low intensity (90 k cycles/sec) sonication to physically separate contaminants from the algae. Brown and Bischoff (1962) have reported that repeated washings and centrifugation of the algae freed of contaminants yield axenic cultures. Attempts to obtain an axenic culture of F. crotonensis using ultrasonic treatment were unsuccessful in this study.

Antibiotics, used singly or in combination, are commonly used to rid algal cultures of bacteria. Axenic cultures of F. crotonensis were obtained via two different antibiotic treatments. The first successful treatment utilized penicillin G, streptomycin sulfate, and chloramphenicol (Hoshaw and Rosowski 1973). The three antibiotics were filter sterilized as a single solution and then added to six flasks containing 50 ml of freshwater "WC" media and 1 ml of unialgal F. crotonensis suspension in exponential growth. The concentrations of antibiotics in each flask are presented in Table 14. Following addition of antibiotics, the cultures were placed under conditions suitable for growth. After 16 hours, three 1 ml aliquots from each culture were plated out on bacterial growth media to detect the presence of bacteria. An additional 1 ml aliquot was aseptically transferred into sterile, antibiotic-free media to maintain the F. crotonensis culture. The plates were then examined weekly for six weeks for bacterial contamination. If no bacterial contamination was found on any of the plates after six weeks, the culture was assumed axenic. Using this technique, axenic cultures of F. crotonensis were established from treatment flasks 1-4. It should be noted that following antibiotic treatment it took approximately six weeks for F. crotonensis to recover from the antibiotic treatment and reach exponential growth phase.

The second successful antibiotic treatment utilized

Table 14. Concentrations (mg L^{-1}) of penicillin G, streptomycin sulfate, and chloramphenicol used for treatment of F. crotonensis cultures.

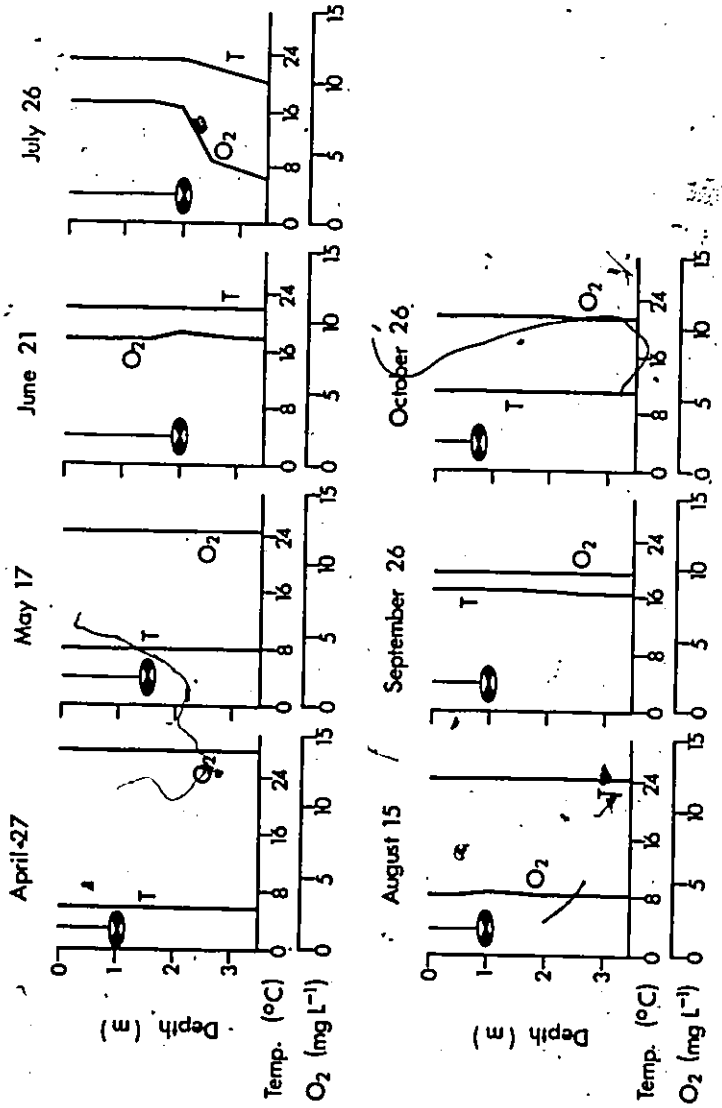
Table 15. Concentrations (mg L^{-1}) of polymixin and streptomycin sulfate used for treatment of F. crotonensis cultures.

Antibiotic	Treatment Flask 1	2	3	4	5	6
penicillin G	505.1	343.1	174.8	88.3	44.3	22.2
streptomycin sulfate	252.6	171.6	87.4	44.2	22.2	11.1
chloramphenicol	50.5	34.3	17.5	8.8	4.4	2.2

Antibiotic	Treatment Flask 1	2	3	4	5
polymixin	58.3	41.7	25.0	16.7	8.3
streptomycin sulfate	75.0	58.3	41.7	33.3	16.7

polymixin and streptomycin sulfate. The two antibiotics were filter sterilized as a single solution and then added to five flasks containing 20 ml of freshwater "WC" media and 5 ml of unialgal F. crotonensis suspension in exponential growth. The concentrations of antibiotics in each flask are presented in Table 15. Following addition of antibiotics the cultures were placed under conditions suitable for growth. After 18 hours, three 1 ml aliquots from each culture were plated out on bacterial growth media to detect the presence of bacteria. An additional 1 ml aliquot was aseptically transferred into sterile, antibiotic-free media to maintain the F. crotonensis culture. The plates were then examined weekly for six weeks. If no bacterial contamination was found on any of the plates after six weeks, the culture was assumed axenic. Using this technique, axenic cultures were established from treatment flasks 4 and 5. Fragilaria crotonensis cultures exposed to the highest concentrations of antibiotics (flasks 1-3) died. As in the previous technique, it took approximately 6 weeks for F. crotonensis to recover from the antibictic treatment and reach exponential growth phase.

9.2 Appendix 2: Oxygen and temperature profiles and Secchi disk measurements recorded at the Pigeon Bay station during the 1983 sampling program.


April 27
May 17
June 21
July 26
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O2
Depth (m)
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O2 (mg L-1)